



Proceedings of the 19th ASPA Congress, Cremona, June 7-10, 2011

To cite this article: (2011) Proceedings of the 19th ASPA Congress, Cremona, June 7-10, 2011, Italian Journal of Animal Science, 10:sup1, 1-138, DOI: [10.4081/ijas.2011.s1](https://doi.org/10.4081/ijas.2011.s1)

To link to this article: <https://doi.org/10.4081/ijas.2011.s1>



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Published online: 24 May 2016.



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ASPA 19th Congress
Cremona, June 7-10, 2011

Italian Journal of Animal Science
2011; volume 10: supplement 1

Agenda for oral presentations

Tuesday June 7 - Animal breeding and genetics - Sala Maffei

- 14.00-14.15 Francesco Tiezzi, Mauro Penasa, Alessio Cecchinato, Christian Maltecca, Giovanni Bittante
Genetic and environmental factors affecting fertility in Italian Brown Swiss cows C-001
- 14.15-14.30 Cristina Sartori, Roberto Mantovani
Effects of inbreeding on fighting ability in Aosta Chestnut and Aosta Black Pied cattle C-002
- 14.30-14.45 Mara Battagin, Mauro Penasa, Martino Cassandro
Flow analysis of Holstein bulls daughters during progeny test C-003
- 14.45-15.00 Ezequiel Luis Nicolazzi, Riccardo Negrini, Raffaele Mazza, Giordano Mancini, Fatima Chegiani, Marco Milanese,
Francesco Filippini, Andrea Albera
Searching for signatures of selection in Italian cattle breeds using the 54k SNP panel C-004
- 15.00-15.15 Licia Colli, Alessandro Achilli, Hovirag Lancioni, Marco Pellecchia, Marcin Rzepus, Giordana Lucente,
Paolo Ajmone Marsan
Phylogeography of the maternal line of European goats by whole mitochondrial DNA sequence analysis C-005
- 15.00-15.30 Alessia Ranieri, Francesca Martuzzi, Antonio Lucio Catalano, Franca Vaccari Simonini
Linear measurements in gait analysis of Bardigiano horses C-006
- 15.30-15.45 Emanuela Tullo, Stefano Biffani, Julio Cesar Alvarez, Rita Rizzi
Genetic variability and population structure in the Carora dairy breed C-007
- 15.45-16.00 Marta Raschetti, Stefania Chessa, Giulio Pagnacco, Denis Guiatti, Misa Sandri, Anna Caroli, Bianca Castiglioni
Association analyses of candidate SNP on meat quality traits in swine C-008

Tuesday June 7 - Dairy production - Sala Mercanti

- 14.00-14.15 Adriana Bonanno, Antonino Di Grigoli, Gabriele Tornambè, Vincenzo Bellina, Francesca Mazza, Massimo Todaro C-009
Effects of nutritional level on lactation response of Girgentana goats with different CSN1S1 genotype
- 14.15-14.30 Salvatore Claps, Maria Antonietta Di Napoli, Anna Rocchina Caputo, Lucia Sepe, Vincenzo Fedele,
Giovanni Annicchiarico, Domenico Rufrano C-010
Sialyloligosaccharides content in colostrum and early milk of two goat breeds
- 14.30-14.45 Antonino Di Grigoli, Alfonso S. Frenda, Gabriele Tornambè, Adriana Bonanno C-011
Effects of grazing Trifolium subterraneum and Lolium multiflorum in various proportions on forage intake and milk production of dairy ewes
- 14.45-15.00 Rami T. Kridli, Abdullah Y. Abdullah, Mohamed Momani Shaker, Ahamad Q. Al-Mamani C-012
Body measurements of Awassi, F1 Charollais-Awassi and F1 Romanov-Awassi ram and ewe lambs

Tuesday June 7 - Animal welfare, health and behaviour - Sala Informatica

- 14.00-14.15 Leonardo Nanni Costa, Diego Magnani, Pietro Calà, Simona Cafazzo, Elisabetta Razzuoli, Massimo AmadoriC-013
Relationship between body temperature and reactivity to backtest in post-weaned piglets
- 14.15-14.30 Grazia Pastorelli, Raffaella Rossi, Paolo Candotti, Carlo CorinoC-014
Evaluation of stress due to mixing in piglets
- 14.30-14.45 Raffaella Rossi, Grazia Pastorelli, Carlo CorinoC-015
Influence of dietary plant antioxidant on oxidative status and immune parameters in post weaning piglet
- 14.45-15.00 Simona Vagni, Luciana Rossi, Carlo Polidori, Francesca Saccone, Loris Giovanni Alborali, Vittorio Dell'OrtoC-016
Multifactorial approach to induce E.coli diarrhoea in weaned piglets
- 15.00-15.15 Luciana Bava, Anna Sandrucci, Alberto Tamburini, Maddalena ZucaliC-017
Effects of season and cow cleanliness on teat apex score and milk somatic cell count
- 15.00-15.30 Roberto Tocci, Clara Sargentini, Alessandro GiorgettiC-018
Morphological, chemical and physical properties on hoof of Maremmano horse, Monterufoli pony and Amiata donkey
- 15.30-15.45 Stefano Schirru, Elisabetta Daga, Antonio Paba, Ilaria Duprè, Laura Mara, Marilia Gallus, M. Francesca Scintu, Roberta ComunianC-019
A survey on the microbiological features of semen from Sarda breed rams

Wednesday June 8 - Animal breeding and genetics - Sala Maffei

- 08.00-09.00 Poster Session
- 09.00-09.45 Jan Thjis van Kaam, Attilio Rossoni, Daniele Vicario, Stefano Biffani, John L. Williams, Paolo Ajmone Marsan, Nicolò P.P. Macciotta, Alessandro NardoneC-020
Genomics in Italian dairy cattle
- 09.45-10.00 Paolo Ajmone Marsan, Ezequiel Luis Nicolazzi, Raffaele Mazza, Lorenzo Bomba, Licia Colli, Riccardo NegriniC-021
Genome wide association studies of dairy production traits in Italian dairy cattle
- 10.00-10.15 Maria Annunziata Pintus, Daniele Vicario, Attilio Rossoni, Jan Thjis van Kaam, Giustino GaspaC-022
Use of a principal component approach to predict direct genomic values for dairy traits in Italian cattle breeds
- 10.15-10.30 Silvia Bongiorno, Susana Bueno, Giordano Mancini, Giovanni Chillemi, Bianca Moioli, Sebastiana Failla, Alessio ValentiniC-023
Deep mRNA sequencing for transcriptome profiling of skeletal muscle tissues
- 10.30-10.45 Coffee break
- 10.45-11.45 Just JensenC-024
How can new technologies for genetic improvement ensure local competitiveness of animal production sector and contribute to global food security?
- 11.45-12.00 Nicolò P.P. Macciotta, Silvia Sorbolini, Giustino Gaspa, Daniele Vicario, Alessio ValentiniC-025
Prediction of direct genomic values for beef traits in Simmental cattle
- 12.00-12.15 Mario Graziano Usai, Tiziana Sechi, Giovanni Battista Congiu, Francis Barillet, Antonello CartaC-026
Using the ovine SNP50 beadchip to detect QTL affecting milk traits
- 12.15-12.30 Tiziana Sechi, Sabrina Miari, Giuliana Mulas, G. Battista Congiu, Annalisa Pernisa, Lia Crasta, Stefania SechiC-027
Detection of polymorphisms on ovine fatty acid synthase (FASN) gene to study their effects on fatty acid composition of milk fat
- 12.30-12.45 Sabrina Miari, Giuliana Mulas, Lia Crasta, Annalisa Pernisa, Antonello CartaC-028
Genetic polymorphisms at intron 4 of ovine stearyl CoA desaturase gene are candidate to affect CLA content in milk
- 13.00-14.00 Lunch
- 14.00-14.15 Donato Matassino, Carmela Maria Assunta Barone, Nadia Castellano, Gianluca Gigante, Michela Grasso, Antonio ZulloC-029
Effect of autochthonous bovine genetic type on some quanti-qualitative characteristics of milk

14.15-14.30	Francesca Ciotola, Sara Albarella, Maria Pia Pasolini, Natascia Cocchia, Luigi Auletta, Vittorio Barbieri, Vincenzo PerettiC-030	<i>Cytogenetic and genetic characterization of an intersex Arabian horse</i>
14.30-14.45	Denis Guiatti, Sandy Sgorlon, Stefania Chessa, Marta Raschetti, Bruno StefanonC-031	<i>Targeting DNA variability in the promoter region of candidate genes to carcass traits of heavy pigs</i>
14.45-15.00	Stefania Dall'Olio, Luca Fontanesi, Luca Buttazoni, Maurizio Gallo, Vincenzo RussoC-032	<i>Single nucleotide polymorphisms in several genes are associated with prolificacy in Italian Large White sows</i>
15.00-15.15	Luca Fontanesi, Giuliano Galimberti, Daniela Giovanna Calò, Emilio Scotti, Pier Luigi Martelli, Raffaele Fronza, Rita Casadio, Vincenzo Russo.....C-033	<i>Genome wide association study for backfat thickness in Italian Large White pigs using a selective genotyping approach</i>
15.15-15.30	Paolo Zambonelli, Roberta Davoli, Silvia Braglia, Luca ButtazoniC-034	<i>Development of molecular markers in pig genes and association with meat pH</i>
15.30-15.45	Roberta Davoli, Silvia Braglia, Paolo Zambonelli, Marco Comella, Roberta CameraC-035	<i>Transcription profile of liver tissue to identify genes related to stress response in pigs of different breeds</i>
15.45-16.00	Maria Gargani, Riccardo Fortunati, Lorraine ParisetC-036	<i>A novel point mutation within the EDA gene involved in X-linked anhidrotic ectodermal dysplasia in Friesian breed</i>
16.00-16.15	Stefano Capomaccio, Nicola Vitulo, Alessandro Albiero, Gianni Barcaccia, Giorgio Valle, Maurizio Silvestrelli, Michela Felicetti, Andrea Verini Supplizi, Katia CappelliC-037	<i>Analyses of exercise transcriptome using RNA-Seq</i>
16.30-16.45	Coffee Break	

Wednesday June 8 - Nutrition and feeding - Sala Auditorium

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09.15-09.30	Alberto Palmonari, Giorgia Canestrari, Nicola Panciroli, Mattia Fustini, Andrea FormigoniC-039	<i>Chemical and nutritional composition of alfalfa at different stages of maturity</i>
09.30-09.45	Andrea Cabiddu, Simona Spada, Margherita Addis, Valeria Giovanetti, Lorenzo Salis, Maria SitziaC-040	<i>Influence of protein fraction content on polyunsaturated fatty acid levels in different forage species</i>
09.45-10.00	Mirko Cattani, Franco Tagliapietra, Matteo Guadagnin, Lucia BailoniC-041	<i>Repeatability of in situ and in vitro digestibility measured at 24 or 48 h of incubation</i>
10.00-10.15	Matteo Guadagnin, Charles Jamie Newbold, Alejandro Belanche, Valerio Bondesan, Lucia BailoniC-042	<i>Effects of soya-bean and rapeseed cake on in vitro rumen fermentation and microbial yield</i>
10.15-10.30	Patrizia Carta, Ignazio Ibba, Antonello CannasC-043	<i>Development of a calibration curve to estimate dairy cattle dietary particle size with NIRS</i>
10.30-10.45	Coffee break	
10.45-11.00	Luciana Rossi, Serena Reggi, Simona Vagni, Corrado Fogher, Antonella BaldiC-044	<i>Evaluation of gastric degradability of antigenic protein expressed in tobacco seeds</i>
11.00-11.15	Sara Minieri, Stefano Rapaccini, Arianna Buccioni, Mauro AntongiovanniC-045	<i>Effect of quebracho tannin on fatty acid profile in rumen solid associated bacteria on soya-bean and linseed oils supplemented diets. An in vitro study</i>
11.15-11.30	Mariella Ferroni, Chiara Riva, Laura Alessandrini, Andrea Arcari, Raffaella Rebutti, Giovanni Savoini, Antonella Baldi, Anna Bernardi, Dario NarducciC-046	<i>Enzyme-based sensor for non-invasive monitoring of ruminal parameters: in vitro results</i>
11.30-11.45	Serena Calabrò, Pier Paolo Danieli, Monica Isabella Cutrignelli, Riccardo Primi, Raffaella Tudisco, Salvatore Del Puglia, Federico Infascelli, Bruno Ronchi, Vincenzo Piccolo.....C-047	<i>In vitro fermentation characteristics of spineless safflower (Carthamus tinctorius L. var. inermis Schweinf) grown under different agronomic conditions in a Mediterranean environment</i>

11.45-12.00	Franco Tagliapietra, Mirko Cattani, Stefano Schiavon.....	C-048
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12.00-12.15	Stefania Colombini, Luca Rapetti, Gianluca Galassi, Luca Malagutti, G. Matteo Croveto.....	C-049
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12.15-12.30	Paolo Berzaghi, Jacopo C. Ferlito, Lorenzo Serva.....	C-050
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12.30-12.45	Guido Invernizzi, Alessandro Agazzi, Andrea Fanelli, Serena Marocco, Mariella Ferroni, Vittorio Dell'Orto.....	C-051
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Wednesday June 8 - Dairy production - Sala Informatica

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09.15-09.30	Federico Vincenti, Stefano Giorgi, Corrado Costa, Francesca Antonucci, Federico Pallottino, Paolo Menesatti, Miriam Iacurto.....	C-066
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09.30-09.45	Miriam Iacurto, Anna Garavaldi, Andrea Palomba, Maurizio Mormile, Federico Vincenti	C-067
	<i>Effect of alternative proteins in the diet on physical-sensory beef quality</i>	
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	<i>Image analysis using Computer Vision System for meat color evaluation</i>	
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11.15-11.30	Claudia Delgadillo Puga, Esteban Mireles Martínez, Mario Cuchillo Hilario, Fernando Pérez-Gil Romo.....	C-073
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	<i>Effect of natural and artificial milk on long chain fatty acid of meat from Sarda suckling lambs</i>	
14.45-15.00	Giuseppe Conte, Evangelia Mourvaki, Marcello Mele, Massimiliano Lanza, Giorgio Formisano, Pietro Pennisi, Luciano Morbidini, Guido Ferruzzi, Pierlorenzo Secchiari	C-076
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Wednesday June 8 - Poultry and rabbit production - Sala Informatica

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	<i>Effect of egg yolk/albumen ratio on hatched chick body composition and broiler meat traits in dual-purpose genotypes by computer tomography</i>	
09.30-09.45	Massimiliano Petracci, Simone Rimini, Gerardo Manfreda, Claudio Cavani, Achille Franchini	C-082
	<i>Effect of free range production system on chicken carcass and meat quality traits</i>	
09.45-10.00	Gerardo Manfreda, Alessandra De Cesare, Massimiliano Petracci, Claudio Cavani, Achille Franchini	C-083
	<i>Effect of free range production system on safety and microbiological parameters of poultry carcasses</i>	
10.00-10.15	Federico Sirri, Adele Meluzzi, Alessandra Roncarati, Paolo Melotti, Roberto Donatini.....	C-084
	<i>Effect of protease supplementation on nitrogen excretion in broiler chicken farming</i>	
10.15-10.30	Silvia Cerolini, Luisa Zaniboni, Chiara Cassinelli, Teresa Maria Gliozzi	C-085
	<i>Sperm DNA fragmentation after cryopreservation in meat type chicken breeders</i>	
10.30-10.45	Coffee break	
10.45-11.00	Abdullah Y. Abdullah, Anas A. Al-Nabulsi, Mohammad A. Jamana'h	C-086
	<i>The effect of cold storage and carcass weight on the shelf life and quality characteristics of whole and deboned broiler breast meat</i>	
11.00-11.15	Alessandro Dal Bosco, Cecilia Mugnai, Silvia Ruggeri, Cesare Castellini	C-087
	<i>Adaptability of different poultry genotypes to organic system</i>	

Thursday June 9 - Animal breeding and genetics - Sala Mercanti

08.00-09.00	Poster Session	
09.00-09.15	Nicola Tormen	C-088
	<i>The trichological analysis in the study of local sheep breeds</i>	
09.15-09.30	Elia Pintus, Silvia Sorbolini, Nicolò P.P. Macciotta	C-089
	<i>Use of locally weighted scatterplot smoothing (LOWESS) regression to study genome signatures in Piedmontese and Italian Brown cattle breeds</i>	
09.30-09.45	Alessandro Crovetto, Lisa Nardi, Riccardo Bozzi	C-090
	<i>Genetic diversity of five Italian sheep breeds</i>	
09.45-10.00	Fabio Pilla, Elena Ciani, Elisabetta Castellana, Emiliano Lasagna, Mario Graziano Usai, Mariasilvia D'Andrea, Progetto Italiano Biodiversità Ovini.....	C-091
	<i>Genetic diversity of seven Italian sheep breeds as inferred by genome-wide SNP analysis</i>	
10.00-10.15	Alessandro Achilli, Hovirag Lancioni, Matteo Bianchi, Marco Mariotti, Lorraine Pariset, Francesco Panella.....	C-092
	<i>A phylogenetic overview of three Italian Merino-derived breeds from a mitochondrial DNA standpoint</i>	
10.15-10.30	Emiliano Lasagna, Simone Ceccobelli, Francesco Panella, Francesca Maria Sarti	C-093
	<i>Pagliarola population or Appenninica breed: a microsatellite discrimination</i>	
10.30-10.45	Coffee break	
10.45-11.00	Marco Tolone, Salvatore Mastrangelo, Baldassare Portolano.....	C-094
	<i>Genetic structure and assignment test in five sheep breeds reared in Sicily using microsatellites</i>	
11.00-11.15	Salvatore Mastrangelo, Maria Teresa Sardina, Valentina Riggio	C-095
	<i>Genetic diversity and phylogenetic relationships among four breeds reared in Sicily using β-lactoglobulin promoter region polymorphisms</i>	

11.15-11.30	Sara Casu, Sotero Salaris <i>A Fortran program to optimize selection schemes in dairy sheep</i>	C-096
11.30-11.45	Stefania Sechi, Sotero Salaris, Ciriaco Ligios, Nicoletta Ponti, Gilles Foucras, Sara Casu <i>Genetic parameters for antibody response to <i>Mycobacterium avium</i> spp. <i>Paratuberculosis</i> (MAP) in natural infected flock</i>	C-097
11.45-12.00	Fiorella Sbarra, Roberto Mantovani, Andrea Quaglia, Giovanni Bittante <i>Genetic parameters of performance tested traits in Maremmana and Podolica breeds</i>	C-098
12.00-12.15	Corrado Dimauro, Roberto Steri, Elia Pintus, Massimo Cellesi <i>Efficiency of the partial least-squares genotype imputation method for dense SNP marker maps in cattle</i>	C-099
12.15-12.30	Georgios Theodorou, Chiara Pecorini, Raffaella Rebucci, Francesca Saccone, Cristina Lecchi, Eleonora Fusi, Ioannis Politis, Luciano Pinotti <i>Effect of hormones and growth factors on cell proliferation and expression of urokinase related genes in a bovine mammary epithelial cell line</i>	C-100
12.30-12.45	Elena Ciani, Ingrid Alloggio, Elisa Pieragostini <i>Evidence of HBAY and HBBA^{Zebu} globin genes in Grey Alpine cattle</i>	C-101
13.00-14.00	Lunch	

Thursday June 9 - Nutrition and feeding - Sala Maffei

08.00-09.00	Poster Session	
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10.30-10.45	Coffee break	
10.45-11.45	Paul Crosson <i>Whole farm systems modelling of greenhouse gas emissions from livestock production systems</i>	C-103
11.45-12.00	Giuseppe Bertoni, Andrea Minuti, Alan Parlatore, Erminio Trevisi <i>Survey on the contribution of animal origin foods in Italian dietary habits</i>	C-104
12.00-12.15	Alberto Tamburini, Matteo Guerci, Chiara Penati, Luciana Bava, Maddalena Zucali, Anna Sandrucci <i>Environmental impact of milk production in intensive farming systems: LCA approach</i>	C-105
12.15-12.30	Luca Rapetti, Stefania Colombini, Mario Pirondini, Chiara Penati <i>Intestinal digestible protein balance and milk urea in lactating goats</i>	C-106
12.30-12.45	Giorgio Dalla Montà, Paolo Carnier, Luca Carraro, Luigi Gallo, Stefano Schiavon <i>Effect of low protein diets on growth performance, backfat thickness and N excretion of heavy pigs</i>	C-107
12.45-13.00	Elisa Marchiori, Enrico Sturaro, Maurizio Ramanzin <i>Wild red deer (<i>Cervus elaphus</i> L.) grazing may seriously reduce forage production in mountain grasslands</i>	C-108
13.00-14.00	Lunch	

Thursday June 9 - Dairy production - Sala Maffei

14.00-14.15	Denis Pretto, Martino Cassandro <i>Analysis of different milk payment systems in Veneto region dairy industries and effect on economic value of milk quality traits</i>	C-109
14.15-14.30	Claudio Cipolat Gotet, Ilario Bazzoli, Massimo De Marchi <i>Influence of the storage of milk on cheese yield</i>	C-110
14.30-14.45	Alessandro Pistoia, Piera Poli, Laura Casarosa <i>Pecorino cheese quality as affected by milk storage time</i>	C-111

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- 09.15-09.30 Filippo Faccenda, Emilio Tibaldi, Giuliana Parisi, Oreste Franci, Fernando LunelliC-128
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- 09.30-09.45 Simone Serra, Giovanna Chessa, Sara Saba, Marco Trentadue, Silvana Manca, Fabrizio Chessa, Nicola Fois, Antonio PaisC-129
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- 09.45-10.00 Rosanna Floris, Silvana Manca, Laura Mura, Nicola FoisC-130
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- 09.30-09.45 Luisa Antonella Volpelli, Adelaide Cino, Michele Comellini, Maria Cristina Ielo, Giovanna Minelli, Domenico Pietro Lo FiegoC-150
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ASPA 19th Congress

Cremona, June 7-10, 2011

Italian Journal of Animal Science

2011; volume 10: supplement 1

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PREFACE

The 19th Congress of the Animal Science and Production Association (ASPA), is held in the capital of one of the main European regions for animal production: Cremona. Despite its Gallic origin (Carra = rock), the name Cremona means *big cream* in modern Italian, in a clear allusion to milk.

In this Congress, 153 oral presentations and 165 posters are presented, for a total of 318 scientific contributions.

The Congress sessions with their relative number of papers are listed as follows: Animal breeding and genetics 87, Nutrition and feeding 57, Dairy production 40, Meat production 46, Animal welfare, Health and behaviour 37, Poultry and rabbit production 28 and Aquaculture 23.

I would like to point out that 4 main lectures will be presented in the Animal breeding and genetics and Nutrition and feeding sessions (two in each), and that the Consiglio per la Ricerca in Agricoltura (Agricultural Research Council), the Agricultural Ministry research agency, which took charge of organizing the Congress, will award a prize for the best work here presented by young researchers.

I would thank, first of all, Prof. Paolo Cescon, President of the Congress, for the contribution of all the CRA in organizing this event. I would also thank all the members of the Scientific Committee, dr. Riccardo Aleandri (President), dr. Fabio Abeni, dr. Fabiola Canavesi, dr. Giacinto Della Casa and dr. Marisanna Speroni, and of the Organizing Committee, dr. Giovanni Lo Piparo (President), dr. Stefania Barzaghi, dr. Maurizio Capelletti, dr. Giorgio Giraffa and dr. Luciano Migliorati. A special thanks to the secretary components, Maria Dissegna and Patrizia Re, assisted by Claudia Federici, Francesca Petrera, Miriam Odoardi, Maddalena Carli, Caterina Sanna, Stefania Marruso, Leonardo Boselli, Milena Rosi and Aldo Dal Prà. A special acknowledgment to Giacomo Pirlo, the real driving force organizing the Congress, a tireless and enthusiastic embroiderer of opportunities in scientific and technological applications. Indeed, I would like to congratulate and thank all those who have organized the meeting and have collaborated in reviewing the summaries for what they have done for ASPA and the Italian Animal Science.

Finally, I acknowledge the institutional agencies, in particular the Ministry of Agricultural, Food and Forestry Policies (MIPAAF), the Regione Lombardia and the Comune di Cremona, and the main sponsors, namely De Laval, SIS NIR and Consorzio Agrario.

Prof. Giuseppe Pulina
President of Animal Science and Production Association



C-001

Genetic and environmental factors affecting fertility in Italian Brown Swiss cows

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The aim of this study was to estimate genetic parameters for fertility and production traits in the Brown Swiss population reared in the Alps (Bolzano-Sud Tirol province, Italy). Fertility indicators were: interval from parturition to first service and interval from first service to conception, either expressed as number of days (iPF and iFC, respectively), or as number of potential 21-d estrus cycles (cPF and cFC, respectively); number of inseminations to conception (Ins); and success at first service (sFS). In addition, relationships between fertility and production traits were estimated. These were: peak milk yield (pMY), average protein content (aPP), average fat content (aFP), actual lactation length (aLL), and actual lactation milk yield (aMY). Data included 71,556 lactations from 29,582 cows reared in 1835 herds. Animals calved from 1999 to 2007 and were progeny of 490 AI bulls. Gibbs sampling and Metropolis algorithms were implemented to obtain (co)variance components in censored threshold and linear sire models, both univariate and bivariate. All the analyses accounted for the fixed effects of parity and year-month of calving, and random effects of herd, cow and sire of the cow. For all the fertility traits heritabilities ranged from 0.055 for sFS to 0.071 for cPC. Estimates of genetic correlations between fertility traits were moderate to strong (0.24 between Ins and cPF, 0.97 between iPF and cPF). Genetic correlations between fertility and production traits confirm the genetic antagonism with values ranging from -0.26 (cPC-IPP) to 0.76 (cPC-aLL). Although reproductive traits are lowly heritable the contemporary inclusion of various fertility traits in a merit index could help to improve reproductive performance of dairy cows.

C-002

Effects of inbreeding on fighting ability in Aosta Chestnut and Aosta Black Pied cattle

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Aosta Black Pied (ABP) and Aosta Chestnut (AC) are dual-purpose cattle autochthonous of West Alps and famous for a strong temperament revived in traditional competitions. The present study aimed to investigate the influence of inbreeding on breeding values for fighting ability (FA) achieved on participants. Data

(n=23,998) of 8259 cows competing in years 2001-2009 were analysed to find out breeding values for FA. A placement score was chosen as phenotype for FA and the linear model included both permanent environment and additive genetics as random effects. A heritability of 0.08 was thus estimated via REML method, and positive variations of the breeding values (EBVs) over years (+1.8%/year for ABP and +3.6%/year for AC) were observed despite a lack of selection for FA. A pedigree analysis carried on the whole registered animals (27,638 and 106,061 records for ABP and AC, respectively) showed an increase of inbreeding over years. The trend resulted greater for ABP cattle (+0.06%/year) than for AC (+0.03%/year), that presented also a greater F value (0.03 vs 0.01 for ABP and AC, respectively) and average relatedness among individuals (AR=0.01 vs AR<0.01 for ABP and AC, respectively) in 2009 born. Including alternatively F or AR within the genetic model didn't change the heritability estimates for FA, although they were both significant in the preliminary ANOVA. Moreover, linear analyses carried on the 33 major lineages to which most of participants belonged (n=6087) revealed an overall negative trend of EBVs as respect to the increment of either F or AR. The incidence resulted greater in ABP (-23 of slope for F and -51 for AR, P<0.01 in both cases) than in AC (b=-18, P<0.05 for F and -47, P<0.01 for AR), resembling the different levels of inbreeding of the two populations. Hence, there seems to be an inbreeding depression also in the behavioural trait of both ABP and AC cattle and its knowledge should be relevant for breeding management.

C-003

Flow analysis of Holstein bulls daughters during progeny test

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The aim of this study was to analyse the flow of daughters of Holstein bulls during the progeny test. Data were provided by the National Breeders Association of Italian Holstein Friesian (ANAFI). For each bull were calculated the distribution of daughters during its progeny test period, as a cumulative frequency of daughters, starting from 21 months, as minimum age at which a bull might have progeny. Data were on about 5600 bulls born between 1986 to 2005. The averages of the ages per bulls, at exit from the Genetic Centre of ANAFI was 11.5 (± 1.3) months, while the age at their first official genetic evaluation was at 59.9 (± 3.8) months. Variables analyzed with ANOVA, using the procedure of SAS (2009), were the cumulative frequencies at 24, 36, 48 and 60 months. The model included the effects of year of birth (20 levels), Artificial Insemination stud (6 levels) and effect of the sire of bull (236 levels). All effects were statistically significant for cumulative frequency of their daughters at 24, 36, 48 and 60 months of age of the bull.

C-004**Searching for signatures of selection in Italian cattle breeds using the 54k SNP panel**

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The recent availability of the bovine 54K SNPchip panel has allowed the investigation of patterns of diversity along the chromosomes, in search for signatures left by past and recent selection. We searched for common extended haplotype homozygosity (EHH) across breeds in 2682 bulls belonging to five Italian breeds, two dairy (Italian Holstein and Brown), one dual purpose (Simmental), and two beef (Marchigiana and Piemontese) investigated within the Italian SELMOL project. The aim of this study was to evidence selection in genomic regions common across-breeds by identifying haplotypes with outlier homozygosity compared to what expected under the neutral drift model. After discarding haplotypes with low frequency (30%), a total of 7283, 7598, 6425, 5948 and 5545 haplotypes were retained in Holstein, Brown, Simmental, Marchigiana and Piemontese breeds, respectively. Less than 200 haplotypes were found significant at $P < 0.015$ in each breed, with 28 regions showing evidence of selected haplotypes in a same region in more than two breeds and 16 regions with significant selection signatures in two different breeds at a distance lower than 500kb. Among these, 5 are in common between Holstein and Brown and 8 between Marchigiana and Piemontese and likely contain genes or regulatory sequences important for milk and meat production, respectively.

C-005**Phylogeography of the maternal line of European goats by whole mitochondrial DNA sequence analysis**

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The molecular phylogeny of livestock species traditionally relied on the characterization of short fragments of the mitochondrial DNA (mtDNA) control region (d-loop); anyway, recent evidence in cattle showed that a far more precise picture can be obtained from mtDNA complete sequences. So far, the analysis of goat (*Capra hircus*) d-loop data highlighted the presence of 6 different haplogroups, whose variability has a lower geographic structure compared to other livestock species.

During this study we analyzed complete mitochondrial genomes to provide a more comprehensive molecular phylogeny of domestic goats. A total of 980 *C. hircus* from western Eurasia and 1 wild goat (*Capra aegagrus*) from Iran were analyzed. The haplogroup affiliation of each specimen was determined by sequencing 1006 bp of the mitochondrial d-loop (np 15343-16348 of the Genbank goat reference sequence, GRS) and comparison to reference data. Among the 352 different control region haplotypes scored, more than 50 were selected for the complete mtDNA sequencing, performed either by direct (Sanger) or high-throughput sequencing (Illumina Genome Analyser Iix, GAIix). The reconstruction of a Median-joining network based on d-loop sequences revealed the presence of haplogroups A and C only, the former being the most frequently found within Italy and Europe, while the topology of the Maximum Parsimony tree obtained from complete mtDNAs (with *Capra falconeri* as outgroup) reveals well defined monophyletic clades corresponding to haplogroups A, B, C and G (divergence times between 40 and 15 Kya) and the occurrence of a recent bottleneck followed by a population expansion, which likely represents the domestication event.

C-006**Linear measurements in gait analysis of Bardigiano horses**

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In kinematics analysis, motion is described quantitatively by linear measurements. Selection in Bardigiano Horse (BH) aims at comfortable gaits for harness and saddle use: objective evaluation of motion patterns is needed. This study compares kinematics analysis data with judge scores (JS) at Performance Test (PT). Walk and trot on sandy ground of thirty eight BH mares and stallions were considered (age 2.5-3.0 years; withers height 140.8±2.4 cm; BCS 2.8-3.0). Tests were performed before and after PT training. Fourteen retro-reflecting half-spheres were glued at defined skeletal reference points. Each BH was led perpendicularly to a video digital camera, sampling frame rate 50 Hz, 7 m from BH left side. S.I.M.I. Motion System analysed the parameters: Stride Length of forelimb and hindlimb (SL F, SL H) distance between successive placements of the same limb; Overtracking (F/H) distance between footfalls of ipsilateral limbs; Forelimb max. height

of carpus (H Carpus swing); Anterior and Posterior phase of F and H limb (F ant, F post, H ant, H post). Data were analysed by SAS. The ANOVA model is: $Y_{ijkl} = \mu + A_i + P_j + R_k (P_j) + H_l (A_i) + e_{ijkl}$ where y =linear measurements; μ =mean; A =year; P =test; R =Replications; H =horse; e =residual error. The solution for each parameter created a horse ranking or index (I) then correlated by SPSS 17.0 with the JS. At walk "Saddle, Impulse-Balance, SL-Elasticity-Agility-Regularity" JS are positively correlated with "SL F, SL H, F Ant, and H Ant" I. F Ant and H Ant I represent the real stride extension. "Jump and Harness" JS are low correlated with all I. Overtracking I is positively corr. with "Imp.-Bal. and SL-Elast.-Ag.-Reg." JS: more H limb covers the footprint of F limb, more impulse the gait has. At trot "H Carpus swing" I is negatively corr. with "Saddle, Imp.-Bal., SL-Elast.-Ag.-Reg. and General quality" JS: higher carpus is lifted, lower the gait quality. This kind of analysis can improve with objective measurements gait evaluation.

C-007

Genetic variability and population structure in the Carora dairy breed

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The genetic structure of the Carora Breed, a Venezuelan dairy cattle population, was studied through the analysis of the whole pedigree. The database included 3506 living animals (38 bulls and 3438 cows), plus their ancestors, for a total of 9320 animals (738 bulls and 8582 cows) born from 1956 to 2009 and reared in 118 herd in the central-western part of Venezuela. All the analysis were carried on with ENDOG v.4.6. The effective number of founders (fe), the effective number of ancestors (fa), individual inbreeding (F), average relatedness (AR) and the rate of inbreeding (F) per generation were analyzed to describe the genetic variability of the population. The Carora pedigree resulted quite complete, the percentage of known ancestors was higher (99%) in the first generation and decreased from 97 to 73 in the following four generations. The maximum number of traced generation, the mean maximum generation, the mean complete generation, and the mean equivalent generation were respectively 13, 6.66, 2.15, and 3.78. The number of founders was 1416, 40 of which may be considered effective founders, while the effective number of ancestors was 30 but those that contributed to the 50% of the total variability were only 11. In the living population the average relatedness coefficient (AR) was 5.88% while the mean inbreeding coefficient (F) was 3.62% and the rate of inbreeding (F) was 0.83%. The generation interval, calculated following the four gametic pathways: sire

to son, sire to daughter, mother to son and mother to daughter, on average resulted 5.98 years. The analysis of the distribution of the herds showed most of them are multipliers, where breeders use purchased males. The inbreeding rate lower than 1% indicates that the Carora population is not alarming; however, monitoring of inbreeding must be maintained.

C-008

Association analyses of candidate SNP on meat quality traits in swine

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An important aim of Italian pig selection is to obtain animals having high aptitude for PDO dry-cured ham production. The efforts of the last years are directed to SNP identification for future large scale association studies. Here five SNP in three candidate genes for meat quality (*CRADD*, *PTPRD*, *PIK3R2*) were found analysing twenty-two animals representing the extreme tails of the distribution for three phenotypes (muscle compactness, fat thickness and the principal component 1) of 231 Large White x Landrace individuals. The principal component 1 was calculated considering nine phenotypes (muscle compactness, marbling, colour uniformity, fat covering, colour, dorsal fat thickness, ham fat thickness, vein system and final score) and was correlated ($P < 0.0001$) with seven of them with absolute correlation values ranging from 0.37 to 0.91. GLM procedure was used for association analysis with meat quality traits. A *CRADD* [A/G] SNP showed a significant association with muscle compactness ($P = 0.0498$), whereas a *PTPRD* [G/T] SNP and a *PIK3R2* [C/T] SNP with muscle compactness ($P = 0.0195$ and $P = 0.0014$, respectively) and backfat thickness ($P = 0.0265$ and $P = 0.0087$, respectively). The SNP were also tested in more than 500 animals belonging to three Italian breeds (Large White, Duroc, Landrace), with extreme EBVs for fat or growth traits. The *PIK3R2* [C/T] SNP showed significant associations ($P < 0.01$) with backfat thickness, average daily gain, feed conversion rate and lean cut yield in Landrace, whereas the *PTPRD* [A/G] SNP showed a significant association with thigh weight ($P = 0.0251$) in Large White.

Acknowledgement

The research was supported by the SelMol project (MIPAAF).

C-009

Effects of nutritional level on lactation response of Girgentana goats with different *CSN1S1* genotype

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Goat polymorphism at $\alpha s1$ -casein loci (*CSN1S1*) influences milk yield and composition. Milk of goats with strong alleles associated to high $\alpha s1$ -casein shows higher fat and casein, longer coagulation time, firmer curds and variation in fatty acids than milk from goats with weak alleles linked to low $\alpha s1$ -casein. Since these milk properties are also affected by nutrition, the aim of this study was to investigate the impact of nutritional level on milk production traits of Girgentana goats with different *CSN1S1* genotype. From a group of goats genotyped using PRC protocols at DNA level, 12 goats having the same genotype at $\alpha s2$, β and κ -casein loci and differing for *CSN1S1* genotype were selected: 6 were homozygous for strong alleles (AA) and 6 heterozygous of strong and weak alleles (AF). Within each genotype, goats were divided into 3 sub-groups based on days of milk (50 or 120 d), housed individually and fed ad libitum with 3 diets in a 3x3 Latin square design with periods of 21 d (14 d for adaptation, 7 d for measuring and sampling). The diets with different nutritional levels were as follows: fresh sulla (*Hedysarum coronarium L.*) (FS); fresh sulla and 800 g/d of barley (FB); sulla hay and 800 g/d of barley (HB). The milk yield increased passing from FS to HB and FB (1352, 1423, 1664 g/d; $P < 0.01$). The effect of genotype on milk yield was linked to the nutritional level, since the milk from FB was higher by 350 g/d in AA and 200 g/d in AF goats. In both genotypes, barley supplement reduced milk fat (3.6, 3.2, 3.0% in FS, FB, HB; $P < 0.01$) and urea (35, 32, 31 mg/dL in FS, FB, HB; $P < 0.01$), and fresh sulla increased casein content (2.7, 2.7, 2.6% in FS, FB, HB; $P < 0.01$). The AA genotype did not increase the casein percentage, but AA milk showed longer coagulation time (r: 16 vs 13 min; $P < 0.01$) and higher curd firmness (a30: 35 vs 29 mm; $P < 0.01$) than AF milk. In this study, *CSN1S1* genotype showed to interact with nutritional level only for milk yield.

Acknowledgement

The research was funded by MIUR, project 2007B4JBWN_002.

C-010

Sialyl oligosaccharides content in colostrum and early milk of two goat breeds

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Milk oligosaccharides provide numerous important biological functions, such as prevention of pathogen binding to the intestinal epithelium and nutritive source for beneficial bacteria. Aim of this work was to study comparatively the variation of oligosaccharide contents in two stages of lactation in two goat breeds. Individual samples from fresh milk and colostrum were obtained from 10 Maltese goats (M) and 8 Garganica goats (G). Each goat provided one sample from the following stages: Colostrum 0h (immediately after kidding); Colostrum 24h; early milk (d 7). The samples, from the morning milking, were immediately frozen at -20°C , lyophilized and homogenized. 3'-sialyllactose (3'SL), 6'-sialyllactose (6'SL) and disialyllactose (DSL) content (mg/L) were analyzed by HPLC. Statistical analysis was carried out by one-way ANOVA (SAS 9.1.3). The differences among breeds were analysed by Duncan multiple range test. The results clearly showed a breed effect at each lactation stage (colostrum 0 and 24h, early milk) on the content of the three oligosaccharides. The colostrum of G breed showed the highest content of 3'-SL both at 0h (253.9 vs 201.3 mg/L) and at 24h (328.5 vs 249 mg/L) and the highest of 6'-SL (174.3 vs 136.9 mg/L at 0h and 200.9 vs 144.1 mg/L at 24h). DSL showed the highest value at 0h (197.9 vs 104.9), and 24h the lowest content (126.4 vs 228.1 mg/L). Also in early milk G breed showed the highest content of 3'-SL and 6'-SL but the lowest content of DSL (94.5 vs 153.2 mg/L). In conclusion, the content of sialyl oligosaccharides changed during the first seven days of lactation with significant differences between the goat breeds.

Acknowledgement

Research carried out within the project GENZOOT (D.M. 12369/7303/08) "Biodiversity and prebiotic characteristics of goat milk", supported by the Ministry of Agricultural, Food and Forestry Policies.

C-011

Effects of grazing *Trifolium subterraneum* and *Lolium multiflorum* in various proportions on forage intake and milk production of dairy ewes

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The aim of this research was to evaluate the effects of grazing *Trifolium subterraneum* (T) and *Lolium multiflorum* (L), as pure or associated crops, on forage intake and milk yield and composition in Comisana ewes. The four following fenced subplots, each of 2500 m², were used for grazing: T, 100% of surface; L, 100% of surface; TL, T and L on 66.6 and 33.3% of surface; LT, T and L on 33.3 and 66.6% of surface, respectively. Each subplot was divided into two parcels in order to have two replicates. Forty ewes were divided into eight homogeneous groups, with regard to milk yield, days in milk and live weight. In spring for 70 days, each group grazed daily for 8 h one of the fenced parcels. Measurements, sampling and analyses regarded the available forage, dry matter (DM) intake (assessed by the n-alkane technique) and milk yield of ewes. Data were analysed for the effect of grazed forage using the GLM procedure of SAS. The clover produced a higher amount of biomass than ryegrass, due also to the higher ryegrass intake of the ewes. Herbage DM intake was lower for the L group than the other groups (1090 vs 1552, 1520 and 1427 g/d for L, T, TL and LT, respectively; P<0.01). Consequently, the diet affected milk yield which was lower for L ewes than for T, TL and LT groups (740 vs 924, 964 and 898 g/d; P<0.05). The exclusive use of ryegrass in the diet led to a tendency to decrease protein, casein and urea contents in milk. Moreover, L milk was higher in curd firming time (k₂₀: 3.39, 1.68, 1.85 and 2.20 min for L, T, TL and LT, respectively; P<0.05) and lower in curd firmness (a₃₀: 20.4, 40.3, 42.6 and 35.2 mm for L, T, TL and LT, respectively; P<0.05). In conclusion, the use in any proportion of *Trifolium subterraneum* showed to increase both DM intake and milk production of dairy ewes.

C-012

Body measurements of Awassi, F1 Charollais-Awassi and F1 Romanov-Awassi ram and ewe lambs

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The aim of this experiment was to study growth performance and body measurements of three genotypes of ram and ewe lambs; Awassi (A), F1 Charollais-Awassi (CA), and F1 Romanov-Awassi (RA). Ten ram lambs and 10 ewe lambs of each genotype were randomly selected to be used in this study. Ram and ewe lambs were kept in separate groups from the beginning until the end of the experiment. Birth weight and weaning weight (70 days of age) were recorded for all animals. Body weight, body dimensions, and body condition scores were recorded at monthly intervals between 6.5 and 16 months of age. Body dimensions were correlated with age, weight, sex, and testosterone concentration. The overall mean square for biological growth parameters including body weight, wither height (H1), rump height (H2), diagonal body length (DBL), body circumference (BC) and hand girth (HG) were significantly different (P<0.05) between the two sexes. Hip height (H3) and body condition score (BCS) were not significantly different. Results of the present study indicate that crossbreeding Awassi with Charollais tends to improve characteristics of meat animals.

C-013

Relationship between body temperature and reactivity to backtest in post-weaned piglets

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Tests simulating an acute stress such as immobilization have been developed for identifying individual reaction patterns related to different coping style in pig. In the Backtest piglets (BT) are immobilized in supine position and categorized as "high-resisters" (HR) and "low-resisters" (LR) on the strength of the escape attempts. It is well known that stress induces vascular changes in different parts of the body, which influence skin and rectal temperatures. Infrared Thermography (IRT) can measure pig's skin temperature detecting, without contact, the thermal energy emitted by the body. The aim of this study was to investigate, in post-weaned piglets, the relationship between the reactivity to the BT and the body temperature during a restrain stress occurred at weighing. On the basis of the BT carried out at 10 and 17 days of age, 96 HR and 96 LR piglets were selected. At the post-weaning, they were examined in 4 replications of 48 subjects allocated in 12 cages composed by 4 subjects: 4 cages with HR subjects, 4 with LR subjects and 4 mixed (2 LR and 2 HR subjects). Individual weight was recorded 3 times while dorsal and ventral IRT images of each piglet were taken. Rectal temperature was also recorded. Room Thermal-Humidity Index (THI) was calculated. Data were analyzed using a model for repeated measures including the fixed effects of replication (1, 4), weighing within replication (1, 12), BT reactivity (HR, LR), type of box (only HR, only LR, mixed), sex (M, F), the random effect of piglet within replication, and the covariates THI and weight within weighing. The type of box affected significantly the rectal temperature which was higher for HR boxes, while there was not a significant effect of BT reactivity on skin and rectal temperatures. Skin temperature was significantly influenced by the THI of environment.

C-014

Evaluation of stress due to mixing in piglets

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The aim of the present work was to evaluate the effect of mixing on some physiological indicators of welfare in piglets fed or not with a natural antioxidant supplementation. Fifty six piglets of average live weight of 6 kg were allotted to two dietary treatments: control diet (CON, N=28) and diet supplemented with dietary plant antioxidant (1 kg/t feed) titrated at 0.5% of phenylpropanoids, expressed as verbascoside (PPG, N=28). After 30 days of dietary treatments, blood samples from 14 piglets/treatment were analysed for serum haptoglobin (Hp), cortisol, blastization of lymphocytes (BLA), aspartate amino transferase and bilirubin (BIL) concentrations and blood antiradical activity through kit Radicaux libres (KRL) test, "SPIRAL Patent", (Couteron, France). After sampling, inside of the same dietary treatment, the half of animals were mixed with animals coming from another pen. After 2 days corresponding to the period able to develop a dominance hierarchy, blood samples of the new groups of animals were withdrawn for the same blood tests. Data were analysed by a GLM with dietary treatment and mixing as main effects. PPG diet tendentially (P=0.073) increased BLA and reduced (P<0.05) Hp; mixing reduced (P<0.05) BIL and increased BLA (P=0.003). PPG diet (P<0.05) and mixing (P<0.001) increased total antiradical activity in blood, expressed as the time (min) required to reach 50% of maximal hemolysis, referred to the whole blood resistance to free-radical attack. The present trial confirms the efficacy of dietary natural antioxidant evidenced by lower haptoglobin values in the PPG group than control; moreover KRL test has been able to discriminate pig welfare in relation to the stressful event such as mixing whereas the physiological parameters frequently used as indicators failed.

C-015

Influence of dietary plant antioxidant on oxidative status and immune parameters in post-weaning piglet

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The aim of the study was to investigate the effect of dietary plant antioxidant in post weaning piglets on oxidative status and immune parameters. At weaning 80 piglets of 8.1 ± 0.2 kg LW were allotted to two dietary treatments: control diet (C) and diet supplemented with dietary plant antioxidant (750 g/t feed) titrated at 0.5% of phenylpropanoids, expressed as verbascoside (PPG). The diets were administered ad libitum. Piglets weight was recorded at 0 and 56 d and blood samples were taken from 10 piglets per treatment for oxidative status and immunoglobulin titre determination. The assessment of reactive oxygen metabo-

lites (ROMs) in blood serum was performed using the d-ROMs tests (Diacron International[®] s.r.l., Italy). The serum concentrations of immunoglobulin of class G and A were measured using a commercial kit (pig ELISA quantitation kit; Bethyl Laboratories, Montgomery, TX, USA). Data on growth performance were analyzed by one way ANOVA and data on serum parameters were analyzed by one way ANOVA, with the weaning value entered as a covariate (SPSS Inc., Chicago, IL, USA). Piglets fed C diet showed a ADG (386 ± 9 vs 425 ± 11 g/d; $P=0.009$) and final weight (29.6 ± 0.7 kg vs 32.1 ± 0.6 kg; $P=0.011$) than PPG supplemented piglets. No difference was observed in ROMs production between control and treated group ($P=0.115$). Piglets fed control diet showed a lower IgG (7.9 ± 0.7 mg/mL vs 16.9 ± 0.7 mg/mL; $P<0.001$) and IgA concentration (0.6 ± 0.1 mg/mL vs 1.11 ± 0.1 mg/mL; $P=0.029$) than PPG supplemented piglets. These results support the view that the employed dietary plant antioxidant positively influences growth performance and immune parameters in post weaning piglets.

C-016

Multifactorial approach to induce *E. coli* diarrhoea in weaned piglets

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Escherichia coli is a cause of multifactorial enteric disease in weaned piglets. The evaluation of new additives is limited by the variable incidence in the field and by the difficulty to obtain a significant percentage of diarrhoea after experimental infection. The aim of this study was to set up experimental conditions to induce *E. coli* diarrhoea in piglets. Fifteen weaned piglets were randomly divided in control group (CG) and infected group (IG). On day 1 piglets of IG were challenged with $3.7 \cdot 10^8$ UFC of *E. coli* O149 strain by oral route in a single dose, while CG received 5 mL of sterile physiological saline. Animals were fasted for 3 hours before and after challenge and 30 mL of 10% NaHCO₃ solution were individually administered 15 minutes before challenge. IG and CG were fed a diet with an high level of protein for 3 days after infection. Faecal score (0:normal; 1:soft; 2:liquid; 3:watery), faecal colour (3:brown; 2:green; 1:yellow), weight and feed intake were evaluated daily. The effects of challenge were analyzed by a multivariate repeated measures (PROC GLM, SAS System). The challenge reduced significantly consistency and influenced the colour of faeces ($P<0.001$), in fact on day 3 IG showed a 90% of diarrhoea incidence, an increased faecal score

corresponding to 2.35 ± 0.66 and a decreased faecal colour corresponding to 1.6 ± 0.51 . In IG diarrhoea lasted for 3.6 days and it wasn't observed in CG. A significant correlation ($r=-0.89$; $P<0.001$) between faecal colour and consistency was found. The zootechnical parameters were not influenced by challenge. The experimental conditions applied in this study could be used in the evaluation of nutritional strategies to prevent or control *E. coli* diarrhoea in weaned piglets.

C-017

Effects of season and cow cleanliness on teat apex score and milk somatic cell count

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Teat end tissue could change after repeated milkings, resulting in the development of a callous ring around the teat orifice. Factors affecting teat hyperkeratosis include: teat end shape, production level, stage of lactation, lactation number, milking management (especially slow milking and over-milking). Also harsh weather conditions or sudden weather changes can affect the level of teat hyperkeratosis. Somatic cells count in milk is an indicator of udder safety and is influenced by stage and number of lactation, milking procedure, hygiene condition of cubicles and udder, seasonal variations. The aim of the study was to investigate the effects of season and cow cleanliness on teat end condition and somatic cell count (SCC). A sample of 16 dairy farms (80 cows on average) were visited during winter, summer and intermediate seasons (autumn or spring) at evening milking. Hygiene score (Schreiner and Ruegg, 2003) and teat score (Mein *et al.*, 2001) were assessed for each milking cows. Individual SCC and milk quality were obtained from AIA database. Records were Linear Score (LS) per cell count, average Udder hygiene Score (US) and average Teat Score (TS). Two classes of observations were defined on US basis: ≤ 2 or >2 score. All data (2161 observations) were analyzed using analysis of variance (proc GLM, SAS). LS showed very low values but with high variability (2.9 ± 1.7 on average); TS was very good (1.8 ± 0.62). Season had a significant effect ($P < 0.001$) on milk quality (fat and protein) with higher values during winter in comparison with other season; unexpected no effect was obtained on LS. Season also affected TS with higher value during intermediate seasons. US was significantly higher ($P<0.05$) during winter in comparison with other seasons. TS were significantly lower ($P<0.02$) in the first class (based on US) compared to the second one. The study confirmed the great effect of season on milk quality and teat conditions and showed that udder cleanliness had a positive consequence on teat apex condition.

C-018

Morphological, chemical and physical properties on hoof of Maremmano horse, Monterufoli pony and Amiata donkey

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The aim of this paper was the hoof quality evaluation in 3 Tuscan equine breeds: Maremmano horse (MH), Monterufoli pony (MP), Amiata donkey (AD). In this work, on 9 MH, 25 MP and 15 AD, the morphological, the chemical and the physical hoof properties were performed. The crown and the foot plantar circumferences, and the wall (W) and the white line (WL) thickness were evaluated. The W, the WL and the sole (S) hardness (H) was also evaluated, through a shore A portable durometer. The hoof chemical content and the hoof Ca, P, Zn, Cu, Ni, Pb, Se content were determined. Results were calculated using one way ANOVA (SAS, 2003), considering as fixed factor the breed. The crown and the foot plantar circumferences were lower in AD and higher in MH. The AD small foot is in accordance with the asinine species standards, while the MH large foot is correlate with the morphological characteristics of the breed. The crown circumference and the foot plantar ratio in MH and in MP were comparable with Catalano horse standards (1984). The WL thickness was lower in MP and, in accordance with the big skeletal development, the wall thickness was higher in MH. The S and the W H was higher in AD and the WL H is similar in AD and in MH. MP showed lower H in the three hoof regions. AD showed the higher content in Crude Protein. Ca, one of the most important hoof mineral contents, is higher in AD and in MP. AD also showed higher content in P, Ni, Pb and Se. AD and MH showed similar Pb content (2,3 in AD and 2,1 in MH). MP and AD showed similar Ca content. Good foot conformation was observed in all performed individuals. Foot size was conformed with the morphological characteristics of the breeds. The WL thickness was lower in MP. The tight WL promotes the S and W cohesion, and consequently the external pathogens potential resistant. AD showed the higher hoof H, with higher protein content. Ni and Se, essential in small concentration, are potentially toxic. AD seemed tolerate these minerals.

C-019

A survey on the microbiological features of semen from Sarda breed ramsStefano Schirru, Elisabetta Daga, Antonio Paba, Ilaria Dupré,
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The aim of this study was to investigate the microbiological characteristics of ram's semen as very few data are reported in literature on this subject. In assisted reproductive techniques (ARTs), semen contamination may arise from systemic or local reproductive tract infections in donors or the inadvertent introduction of microorganisms during ARTs, and may lead to decrease in viability of spermatozoa, disease transmission and abortion. In this study the fresh semen of 10 Sarda breed rams, known to be healthy and clinically free of parasites, was analysed during the spring (March-April). Semen from two consecutive ejaculates from each ram was collected weekly (for 3 times) and were split into two portions: one remained undiluted, and the other was diluted with skimmed milk extender (1.6×10^9 motile spermatozoa/mL) containing streptomycin, penicillin and sulphanimide. In order to obtain a sufficient amount of semen to be analysed, the rams were divided into 2 groups (A, B) per each group pooling the semen from 5 rams. The undiluted portion was analysed immediately, whereas the diluted was processed 6 h later (max time before use in artificial insemination). Sixty ejaculates were analysed. For both undiluted and diluted samples, the enumeration of total bacteria load, presumptive *Staphylococcus aureus*, *Campylobacter* spp., *haemolytic streptococci*, *Staphylococcus* spp., *Pseudomonas* spp., yeasts, molds, and *Enterobacteriaceae* was performed by plate count. One-way ANOVA was used to statistically test bacterial counts means. Significant differences ($P < 0.05$) between group A and B were found only for some microbial groups. The total bacterial counts were about 7 and 5 log CFU/mL, for undiluted and diluted samples, respectively. Generally, counts before and after dilution were significantly different ($P < 0.05$) showing effectiveness of the extender in lowering bacterial load. However, up to date there are only limited data regarding the dose bacterial agents affecting semen performance in ARTs.

C-020**Genomics in Italian dairy cattle**

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Here we aim to present an overview of the developments in genomic selection in Italian dairy cattle. A major difficulty in the selection of parents is that only half the genetic variance in offspring is accounted for by the variation between parents and the other half depends on Mendelian sampling. Two methods for capturing this Mendelian segregation are performance testing and DNA analysis. DNA methodologies are less time consuming and now also cheaper than performance testing. As a consequence in dairy cattle most countries followed the development of the Illumina Bovine SNP50 BeadChip that contains 54K markers and are now genotyping sires with this or higher density panels. The transition of laboratories to SNP (single nucleotide polymorphisms) technology, made it possible to (1) strongly increase the number of markers collected, (2) automate marker data collection, and (3) decrease the cost of data collection. The SelMol, ProZoo, Elica, Quantomics and Genvia projects, AI centres and international exchanges provided genotypes to Italian breed associations. This resulted in reference data sets based on genotyped progeny tested bulls, which have the most reliable indices. Large reference data sets are the main determining factor for the gain in reliability of indices obtained. Generally, around 40 thousand SNPs are retained after quality selection. In genomic selection, the genetic effects of these SNP markers are predicted simultaneously and the direct genomic values of animals for a trait are obtained. To maximise reliability the obtained indices are combined with traditional indices to a blended genomic index. The first application of genomic indices in breeding is preselection of young bulls and bull dams. Since July 2010 ANAFI provided genomic BLUP and blended indices to AI centres. In Holsteins fat % gained 31% reliability above the pedigree index. Across 31 traits the average gain in reliability was 12%. In 2011 ANAFI and the Italian AI centres intend to close an agreement to join an international consortium. Since November 2010 ANARB computed national genomic BLUP and blended indices for AI centres. In the Brown breed, body condition score gained 11% reliability above the pedigree index. Across 9 traits the average gain in reliability was 5%.

ANARB joined the Intergenomics program whereby Interbull will compute direct genomic values for all participating countries from spring 2011. In 2011 ANAPRI will implement genomic BLUP using principal components in order to reduce the number of effects to estimate. Furthermore ANAPRI is sharing genotypes with Simmental populations from Germany and Austria in 2011. For milk in Simmental, the correlation between genomic and traditional breeding values is 0.62. Since summer 2010, new SNP chips entered the market. A low density chip will make genomics more affordable. A high density chip can help in gene detection and across-breed genomic evaluation. Using imputation algorithms, identical chromosome segments between relatives can be inferred. DNA is getting a more substantial place in dairy breeding and holds promise for new traits.

C-021**Genome wide association studies of dairy production traits in Italian dairy cattle**

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For many years, dairy cattle breeding programmes have focused mainly on milk production traits. The recent availability of 54K SNP markers spread throughout the genome has allowed the investigation of the DNA variation associated with these phenotypes. Here we describe the results of a genome-wide association analysis (GWAS) of three main production traits (milk, fat and protein yield) in two dairy (Holstein and Brown) and one dual-purpose (Simmental) Italian breeds, analysed within the Italian SelMol project. Dependent variables were de-regressed estimated breeding values (DRPs) for 1042 Italian Holstein bulls, and Daughter Yield Deviations (DYDs) for 749 and 479 Brown and Simmental bulls, respectively. GWAS was run using the R package GenABEL by fitting a linear model over phenotypes pre-corrected by the (genomic) polygenic effect. The significance threshold was corrected for multiple testing by a Bonferroni correction that considered 500 independent linkage blocks ($\alpha = 1 \times 10^{-04}$). Italian Holstein and Brown have the highest number of markers significantly associated to production traits (55, 60 and 40 in Holstein, and 55, 44, 66 in Brown for milk, fat and protein yield, respectively), followed by Simmental (36, 49 and 36 significant markers for the same traits). Only one significant marker was found in common between breeds (ARS-BFGL-NGS-4939 for milk yield, in Holstein and Simmental). Our findings may result from different population genetic structure, selection and evolutionary history of the three breeds analyzed, the low number of samples and the different statistical models used to estimate the dependent variables, among others. Further work will include more samples to increase power of GWAS and to validate our findings.

C-022
Use of a principal component approach to predict direct genomic values for dairy traits in Italian cattle breeds

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A main issue in genomic selection is the huge unbalance between markers and phenotypes. Aim of this work is to use principal component (PC) analysis to reduce predictor dimensionality for calculating direct genomic breeding values (DGV). Bulls of three breeds were genotyped with the 54K Illumina beadchip: 863 Holstein (H), 749 Brown (B), 479 Simmental (S). Edits were on missing data, Mendelian inheritance, MAF (<0.05), deviance from Hardy-Weimberg equilibrium. After the editing, 40,901, 37,254, and 40,179 SNP were retained for H, B and S respectively. PC analysis was used to extract latent variables from retained SNP. For each breed, a reference (older animals with genotypes and phenotypes) and a validation (younger animals with only genotypes) data set were created. PC score effects on polygenic EBV for some dairy and functional traits (Table 1) were estimated on reference bulls using a BLUP model. Estimated effects were used to predict DGV on validation bulls. PC analysis was effective in reducing predictor number (2257, 2466, 2566 PC for B, S and H, respectively). Correlations between DGV and EBV (Table 1) for validation bulls ranged from 0.13 to 0.63. They are low to moderate, but in accordance with other studies with similar population sizes. The use of PC analysis in DGV calculation allowed for a relevant reduction in computational time (<95%) obtaining correlations equal to those obtained using SNP genotypes as predictors.

Table 1. Correlations between DGV and EBV.

	Holstein	Brown	Simmental
Fat yield	0.45	0.32	0.38
Fat %	0.37	0.38	0.13
Milk yield	0.25	0.18	0.47
Udder score	0.47	0.52	0.39
Protein yield	0.39	0.18	0.42
Protein %	0.41	0.51	0.32
Economic index	0.41	0.31	0.28
Somatic cell score	0.63	0.42	0.32

C-023
Deep mRNA sequencing for transcriptome profiling of skeletal muscle tissues

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High-throughput mRNA sequencing (RNA-Seq) is an innovative tool for transcriptomics which permits measurement of differential gene expression. Using RNA-Seq, we aimed at analysing gene expression in muscle RNA of two Italian cattle breeds, Maremmana (M) and Chianina (C), to uncover genes responsible for meat tenderness, an important trait for meat quality. Samples were classified hard and tender on the basis of phenotypic analysis, including Warner-Bratzler shear force, the index of myofibrillar degradation, the proteolytic activity and the insolubility of collagen. Total RNA was extracted from Longissimus dorsi skeletal muscle of young bulls of both breeds. Hard muscle of C vs M and tender muscle of C vs M were compared in two individuals, for each breeds, representing the extreme degrees of tenderness. Single-end libraries were generated using full length cDNAs, so that ditags represent the 5' capped and the 3' polyA tail signatures of individual transcripts. Each cDNA library was sequenced on the GAI Illumina sequencing platform (GAI). Our deeply sampled RNA-Seq generated 19,590,749 short sequence reads. We pre-processed and assayed the reads quality with specialized tools as FastQC and FASTX-Toolkit. We used TopHat to map splice junctions in RNA-Seq reads and Cufflinks to estimate transcript abundances and to test for differential expression and regulation in RNA-Seq samples. Preliminary inter breed comparison of hard muscle RNA revealed 28,451 transcripts (88.3% with known annotation) distributed into 451 significant transcripts corresponding to 318 genes. RNA comparison of tender samples resulted in 30,850 transcripts (87.1% with known annotation) distributed into 592 significant transcripts corresponding to 425 genes. These findings have crucial implications for the design and data explanation of RNA-Seq studies on gene expression variations between individuals of the same or different breeds.

C-024
How can new technologies for genetic improvement ensure local competitiveness of animal production sector and contribute to global food security?

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Increasing population size, too little or too much food per capita, pressure on use of arable land and extensive use of (non) renewable nutrients are very general problems. Do they have anything to do with animal genetics? In my opinion a great deal. Genetics is not the solution to all these problems but can greatly assist in mitigating them. This paper will review current challenges in the global food production system, recent developments in quantitative genetics and genomics, and consider the expected impact on animal breeding programs. The size of the human population is reaching 7 billion during 2011 and is expected to approach 10 billion in 2050. Continued economic development leads to increased individual demands from large population segments. Together, these trends will put considerable pressure on the global food production system. In 2011 more than 1 billion humans are food insecure with a caloric intake that is detrimental to their health. Another billion are on a diet that lack of vital amino acids and minerals etc. A diet that especially in children, are detrimental to physical and mental development. At the other end there are about 1 billion humans that overuse food which leads obesity, impaired wellness, and life style diseases like diabetes. Agricultural production takes up very large parts of available land resources and is also responsible for large emissions of climate gases, contribute to leakage of nutrients such as nitrogen and phosphorus with detrimental effects on aquatic catchments. The current source of phosphorus is primarily limited fossil resources which continued utilization cannot be sustained. Summing up, humankind are increasingly pressuring overall planetary limits requiring rethinking of the global food production system. The increase in global population size must be accompanied by corresponding increases in the efficiency of the food production system. In general, animal based foods are inefficient due to high conversion and maintenance cost compared to plant based food. To ensure future competitiveness of the animal sector future breeding programs must have a continued focus on efficiency of production. Reduction of greenhouse gas emissions and improved feed efficiency are in general favourably related. The technologies available to breeding programs currently are developing at a hitherto unseen pace and in large animal species the expected rate of genetic gain is expected to double over what was seen in the latter part of the 20th century. Especially, the use of genomic information will impact on breeding programs by drastically reducing running cost, but so far the breeding industry has not yet, to any large degree, made any radical changes in their breeding programs.

Genomic information facilitates inclusion of traits that are difficult to select in classical breeding programs. This includes traits such as quality of meat and milk or disease resistance in production animals. This may lead to new and highly specialized breeding programs for local products or to development of breeding stock specially adapted to local circumstances. Current breeding programs have been successful in developed countries that can support a complex infrastructure. Development of new breeding programs based on technology that requires a much smaller scale of operation enables implementing such programs in developing countries with a lesser need for infrastructure. The use of this as a vehicle to provide genetically improved domestic animals to the people who need it the most must be explored.

C-025
Prediction of direct genomic values for beef traits in Simmental cattle

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Genomic selection aims at predicting breeding values by estimating effect of chromosome segments on phenotypes using dense SNP maps. However, the use of high-throughput platforms results in a marked unbalance between genotypes available and number of markers. Principal component analysis (PCA) was able to reduce (<90%) the predictors for calculating of direct genomic values (DGV) in dairy cattle. In the current study, PCA is used to calculate DGV for meat traits in a sample of 457 Italian Simmental bulls genotyped with the 54K Illumina beadchip. PC extraction was carried out on 40,179 edited SNPs, separately for each chromosome, and 2466 PC were obtained (70% of explained variance).

Table 1. Correlations between DGV and EBV.

Trait	R-BLUP	PC-BLUP
Average daily gain	0.451	0.357
Feet and leg score	0.739	0.736
Calving ease direct effect	0.199	0.194
Beef Index	0.657	0.657
Muscularity	0.705	0.707
Size	0.526	0.521
Cow muscularity	0.830	0.831

Bulls were divided into reference and validation data sets,

according to birth year. Effect of PC scores on polygenic EBVs was estimated in the reference population with a BLUP model (PC_BLUP). Traits analyzed were average daily weight gain, size, muscularity, feet and legs, Beef index (economic index), calving ease direct effect, cow muscularity. Accuracy was calculated as correlation between DGV and polygenic EBV in the validation data set. Results were compared to those obtained using the 40,179 markers as predictors (R_BLUP). Muscularity, feet and legs, and the Beef Index showed highest accuracies (Table 1), whereas calving ease had the lowest. No differences were detected between the two methods, except for daily gain where PC_BLUP outperformed R_BLUP.

C-026

Using the ovine SNP50 beadchip to detect QTL affecting milk traits

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A set of 44,936 SNP from the Ovine SNP50 BeadChip (Illumina) was used to identify QTL affecting milk, fat and protein yields (MY, FY, PY), fat and protein contents (FC, PC). The population consisted of 10 Back-Cross Sarda x Lacaune sire families (853 daughters). The analysed phenotypes were the daughter EBVs adjusted for half the dam EBVs. The sire phases were estimated from the daughter genotypes. A within-family linear regression was performed following the model: $y_{ij} = s_i + (2p_{ij}-1)a_i + e_{ij}$, where y_{ij} is the individual phenotype; s_i is the effect of sire i ; p_{ij} is the probability of inheriting one defined QTL allele from sire i for daughter j ; a_i is half the allele substitution effect of the QTL carried by sire i , and e_{ij} is the residual assumed to be normally distributed with heterogeneous variance. The model was tested by likelihood-ratio test (LRT) at each SNP position. The significance thresholds were obtained from the chromosome-wide (CW) and genome-wide (GW) distributions of maximum LRT obtained by 10,000 within-family permutations. The average information content, calculated as $(1-2p_{ij})^2$, was 0.99. On the whole, 30 QTL exceeded the 0.05 CW threshold. Six affecting MY were located on OAR 3, 13, 17, 20 and 25 (2 QTL~27 cM apart); 4 affecting FY on OAR 3, 7, 9 and 25; 5 affecting PY on OAR 3, 7, 13, 17 and 20; 6 affecting FC on OAR 3, 7, 9, 17 and 20 (2 QTL~11 cM apart) and 9 affecting PC on OAR 1 (2 QTL~172 cM apart), 3, 7, 12, 13, 16, 17 and 20. Among these 7 QTL exceeded the 0.05 GW threshold. One affecting MY was located on OAR 3; 1 affecting FY on OAR 3; 2 affecting PY on OAR 3 and 13; and 3 affecting FC on OAR 3, 7 and 17. The number of significant families (LRT>4) ranged from 1 to 5. Allelic substitution effect values ranged from 0.11 to 0.33 sdu. The dense SNP map allowed to confirm QTL pre-

viously detected with a sparse map and to identify new regions affecting milk traits.

Acknowledgement

Work realised in the framework of the European Project 3SR of the FP7.

C-027

Detection of polymorphisms on ovine fatty acid synthase gene to study their effects on fatty acid composition of milk fat

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In a previous study, a genome region significantly affecting the milk fat content of myristic (C14:0) and palmitic acids (C16:0) was detected on ovine chromosome 11 by using a sparse microsatellite map on 10 sire families of Sarda X Lacaune back-cross ewes. Among the genes located in the proximity of the significant peak, Fatty Acid Synthase (*FASN*) was identified as a positional candidate gene since it codes for a multifunctional enzyme complex responsible for the de novo biosynthesis of fatty acids. Furthermore in bovine, previous studies found significant associations between SNPs in *FASN* and variation in the fatty acid composition of adipose and milk fat. Thus, the sequencing of ovine *FASN* gene on the 10 sires was carried out in order to identify polymorphisms. Since only few ovine sequences were available, the bovine gene (41 exons) was used as reference (GenBank accession No. AF285607). So far approximately 42% of the ovine gene has been sequenced. Ten SNPs were identified in the introns: 5380 A>G, 5518 T>G, 5521 G>A, 5549 T>C, 9174 G>A, 10,112 T>C, 13351(*) G>A, 14,696 G>A, 17,286 G>A, 18,496 C>T and one in the 3'UTR: 19,259 C>T. Two synonymous mutations were found in the exons (17,890 C>A and 10,019 C>T). Four mutations (10,024 G>A, 10,659 C>T, 18,743 G>C and 18,748 G>A) determine the substitution of serine with asparagine, arginine with tryptophan, glycine with alanine, and alanine with threonine respectively. SNPs which resulted in heterozygous state in significant sires were considered candidate to explain the within family variation and the genotyping of the daughters is ongoing.

(*) *Rough position on bovine genomic sequence.*

Acknowledgement

Work funded by the program APQ "Attivazione del Centro Biodiversità al servizio dell'allevamento" of RAS.

C-028**Genetic polymorphisms at intron 4 of ovine stearoyl CoA desaturase gene are candidate to affect CLA content in milk**

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A significant QTL affecting the rate of desaturation of vaccenic acid to CLA in milk was found in the region surrounding stearoyl CoA desaturase (*SCD*) gene location in 4 out of 10 half-sibs families of Sarda x Lacaune backcross ewes. On this basis *SCD* gene was sequenced in the 10 sires to find candidate polymorphisms to explain the within family variation. We sequenced 9.2 kb of the whole gene and the obtained consensus sequence was submitted to GenBank Accession n° GQ904712.1. Further 6 kb were sequenced. Five out of 11 already known SNPs and 11 new SNPs were detected. One synonymous SNP was located in the coding region (exon5: 10279C>T) and 4 SNPs were located in non coding regions: 1 in intron 2 (5982 G>A); 3 in intron 4 (9207G>A, 9667A>T and 9883C>T); 3 SNPs were located in the gene promoter region (1017C>T, 1035C>T and 1445C>A) and 3 SNPs were located within the 3'UTR (14,081G>A, 14,260G>A and 14,360A>G). SNPs positions are given according to bovine sequence (GenBank Accession n° AY241932.1) which is available for the whole gene. Candidate SNPs to explain the within family variation were identified by verifying the heterozygous state in the significant sires. No common SNP was found heterozygous in all significant sires. Most SNPs were heterozygous in significant and not significant families. Only the 4 significant sires showed at least one heterozygous SNP at intron 4 (1589 bp). Two sires were heterozygous at 8641A>C, 9207G>A and 9883C>T, one sire was heterozygous at 9667A>T and another one at 10067C>T. On this basis, intron 4 has been considered the most likely candidate portion of *SCD* gene to affect CLA content in milk. Thus, the genotyping of identified intron 4 polymorphism is ongoing on the offspring of the four significant sires.

Acknowledgement

Work funded by the program APQ "Attivazione del Centro Biodiversità al servizio dell'allevamento" of RAS.

C-029**Effect of autochthonous bovine genetic type on some quanti-qualitative characteristics of milk**

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Aim of the study was to investigate the effect of autochthonous genetic type (AGT) and parity order (PO) on milk yield and quality of 77 cows belonging to Agerolese, Bianca Val Padana, Burlina, Cabannina and Valdostana-Castana AGTs. The animals were bred at the Experimental Farm of ConSDABI. The samples for analytical determinations (protein, fat, lactose, minerals, pH and acidity) and somatic cell count were collected monthly for the entire lactation. The data were processed by GLM procedure of SAS with AGT and PO (primiparous, secondiparous, multiparous) as fixed factors. The results showed a significant effect of the AGT on quantity and quality traits of milk: i) Burlina and Agerolese gave respectively, 24 and 25 quintals of milk in 180 days of lactation, 27 and 28 in 210 days and 29 and 30 quintals in 240 days; these productions were significantly higher than Castana ($P < 0.05-0.01$) which was the least productive; ii) Agerolese showed the highest fat percentage in comparison with the other AGTs ($P < 0.001$) except Cabannina; Bianca Val Padana gave the highest protein percentage (3.50, $P < 0.01$), while the lowest levels were observed in Burlina and Agerolese (3.30 and 3.31). In relation to the PO, the results showed a higher production level in multiparous than primiparous ($P < 0.001$) and secondiparous ($P < 0.05$) at 180 and 210 days of lactation, as expected, but at 240 days of lactation the level of multiparous (29.86 quintals) was significantly higher only than primiparous ($P < 0.05$). Moreover, rising PO, fat percentage increased (from 3.50 to 3.54 to 3.76, $P < 0.01$) whereas protein and lactose percentage decreased ($P < 0.001$). Overall, the results confirm that milk composition varies according to the AGT and, in particular, Burlina and Agerolese differ more markedly from the other AGTs.

C-030**Cytogenetic and genetic characterization of an intersex Arabian horse**

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An Arabian foal of 18 months and with stallion appearance was submitted for cytogenetic and molecular genetics examinations due to abnormalities of external genitalia (a small penis with

cranio-caudal direction and an incomplete foreskin) and the presence of ovotestis-like structures in abdominal cavity evaluated with transrectal ultrasound examination. Whole blood was taken for karyotype analysis and to detect the presence of *SRY*, *ZFY* and *ZFX* genes. Through RB-banding the animal showed the typical female equine karyotype ($2n=64, XX$) while no chromosomal abnormalities were found, data confirmed by CBA-banding. Primers used for PCR amplification of *SRY* are: *ECA-SRY-F* 5'-TGCTATGTCCAGAGTATCCAACA-3' and *ECA-SRY-R* 5'-TGAGAAAGTCCGGAGGGTAA-3' (fragment size 714 bp) while those for *ZFY/ZFX* are: *ECA-ZF-F* 5'-AAATCAAACCTTCATGCCAAT-3' and *ECA-ZF-R* 5'-TTCCGGTTTCAATCCATC-3' (fragment sizes 604 bp for *ZFX* and 553 bp for *ZFY*). Thermal cycling parameters were: 94°C for 10 min followed by 35 cycles of 45 s at 94°C, 60 s at 58°C and 60 s at 72°C, a final extension was done at 72°C for 10 min. PCR products were separated on 1.8% agarose gel containing ethidium bromide. Molecular analysis of the *SRY* fragment revealed its absence, while as regard *ZFY/ZFX* detection only the fragment of 604 bp was found indicating the absence of *ZFY* gene and the presence of *ZFX*, typical condition of female equine. The condition found in this work has already been reported previously in the horse and further analysis of genes involved in sex differentiation (like *Sox9*, *Amh* and *Dax1*) are needed to define the causes of intersex in this foal.

C-031

Targeting DNA variability in the promoter region of candidate genes to carcass traits of heavy pigs

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The research was performed to find associations between SNPs of targeted functional genes and porcine carcass traits. Three different breeds were considered: Italian Large White, Italian Duroc and Italian Landrace. The estimated breeding values (EBVs) for average daily gain (ADG), back fat thickness, lean meat, ham weight and feed conversion rate were obtained from the National Association of Pig Breeders of Italy. Within each breed, 200 individuals were selected, half in the higher and half in the lower 5% tail of the normal distribution curve of the population of the variable ADG. DNA was extracted from blood samples and analysed for single nucleotide polymorphisms (SNPs) in promoter regions of 6 genes (*LEP*, *MYF6*, *MYOD*, *OPN*, *PIK3R2*, *SCD*), already known for associations with carcass traits. Allele frequencies were calculated for each SNP locus and all of these resulted polymorphic. Pearson correlation test between EBVs

evidenced significant correlations ($P<0.01$) between all considered phenotypic traits and Chi-square analysis shown significant differences ($P<0.01$) in alleles frequencies between minus e plus variant groups for ADG. Furthermore significant associations between SNPs of genes and productive traits were found. Putative transcription factor binding sites were identified in these regions using bioinformatic tools.

Acknowledgement

The research was supported by the SelMol project financed by MIPAAF.

C-032

Single nucleotide polymorphisms in several genes are associated with prolificacy in Italian Large White sows

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In this study we evaluated the effect of marker genes on prolificacy in Italian Large White (ITLW) purebred sows. Hair roots were sampled from 1803 sows reared in six farms located in the Northern Italy and phenotypic litter size records were used to calculate estimated breeding values (EBV) for number of piglets born alive at first parity (NBA1) using a BLUP-Animal Model. A total of 24 single nucleotide polymorphisms (SNPs) in 23 genes were genotyped. Genes were selected based on their function, and/or position close to quantitative trait loci regions and/or previous studies. Sixteen SNPs showed minor allele frequency (MAF) > 0.05. Association analysis between these polymorphisms and NBA1 EBV was performed using PROC GLM of SAS. SNPs in *growth differential factor 9 (GDF9)*, *glutathione peroxidase 5 (GPX5)*, *CGI-146*, *Pro-Apoptotic Gene Activated During the Early Response to DNA Damage (PNAS-4)* and *retinol binding protein 4 (RBP4)* genes showed significant association ($P<0.003$) with NBA1 EBV. Additional SNPs in four genes (*cytochrome P450, family 21, subfamily A, polypeptide 2, CYP21A2*; *fucosyltransferase 1, FUT1*; *mannosidase alpha class 2 B member 2, MAN2B2* and *transforming growth factor beta type 1 receptor, TGFBR1*) showed suggestive association ($P<0.05$) with the EBVs. Our results provided confirmatory evidences of previously reported studies, and identified novel SNPs associated with litter size. Some of the investigated markers could be useful for marker assisted selection to improve prolificacy in ITLW sows.

C-033**Genome wide association study for backfat thickness in Italian Large White pigs using a selective genotyping approach**

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Fatness is a trait of particular importance for the Italian pig breeding industry that is mainly focused on high quality dry-cured ham production. On the one hand a reduced fatness increases consumers' acceptance of pork and carcass value, whereas on the other hand an appropriate fat coverage of the legs is needed for processing dry-cured hams. Back fat thickness (BFT) is a measure that can be easily recorded and that is included as a target trait in selection programs of Italian heavy pig breeds. In this work we carried out a genome wide association (GWA) study using the Illumina PorcineSNP60K chip to identify chromosome regions affecting BFT in the Italian Large White breed. The genotyped pigs were chosen among a population of about 12,000 Italian Large White animals individually performance tested at the Test Station of the National Pig Breeder Association (ANAS). As experimental design, we used a selective genotyping approach within this population based on the extreme and divergent BFT estimated breeding value (the 150 pigs with most negative and the 150 with most positive estimated breeding values). Using this approach, several chromosome regions affecting BFT have been identified. Some of them overlap with the position of candidate genes and QTL regions already reported in other studies.

C-034**Development of molecular markers in pig genes and association with meat pH**

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The pork pH is an important parameter to assess the quality of fresh and seasoned meat products. Several QTL regions influencing pork pH are reported in PigQTLdb, although up to now only two major genes, *RYR1* and *PRKAG3*, related to pig meat pH have been identified. The aim of this study is to identify in silico SNPs

located in the transcribed portion of genes mapped on pig chromosome (SSC) 1, 2 and 3, in QTL regions for meat pH. A total of 1822 UniGene clusters containing at least four sequences each were selected in the target regions. By aligning the sequences available in each cluster, we found 356 SNPs suitable to be genotyped by the high-throughput Illumina GoldenGate system. These SNPs were utilised to genotype 284 Italian Large White pigs for which meat pH1 and pH2 were measured at slaughter. The SNPs segregating in this population were aligned with pig Sscrofa9.2 genomic sequence using ENSEMBL tools to locate and analyse the corresponding genes and chromosome segments. On SSC1 the genes analysed are located within a 10 Mb interval, on SSC2 in a region of 6 Mb and on SSC3 in 2 Mb. An association study between SNPs and the traits investigated was performed utilizing Plink software. Results highlighted 15 markers significant at P-values between 0.03 and 0.002 after correction for false positive results and multiple testing. Further studies are under way to analyse the significant markers in additional Italian Large White populations. Also, other SNPs are being tested, to better define the chromosome regions where the genes for meat pH variation are located.

C-035**Transcription profile of liver tissue to identify genes related to stress response in pigs of different breeds**

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Stress response in livestock may have negatively effects on growth, reproductive performance, disease resistance and meat quality. The knowledge of genes responsible for stress susceptibility/tolerance are presently very poor. The aim of this study was to compare by microarray analysis the transcription profile of genes expressed in liver tissue of two groups of pigs from different breeds (Italian Large White, ILW; Italian Duroc, ID; Pietrain, PI): a group of pigs was exposed to physical stress before slaughtering and the other one was treated under the usual slaughter procedure. On the whole 34 pigs were utilised. Total RNA was isolated, and the reverse transcribed mRNAs were individually hybridised to the Operon/Qiagen pig 11k Oligo set. Differentially expressed (DE) genes between stressed and not-stressed pigs were found and among breeds different functional categories of genes were detected. In ILW the main DE genes were involved in stress response, cytoplasm/intracellular organelles activities and enzymatic activities, in ID samples genes for stress response, cellular homeostasis processes and cellular regulation processes were mostly represented. In PI (*RYR1* g.1843CC) we found DE genes involved in cellular regulation processes and in PI (*RYR1* g.1843CT) the main functional categories of the DE genes belong

to metabolic processes regulation and stress response. The differences among breeds will be analysed in deep in order to know the gene networks involved in stress response in the different breeds. The final goal will be finding stress-related genes to be used as markers for association studies with meat quality.

C-036

A novel point mutation within the *EDA* gene involved in X-linked anhidrotic ectodermal dysplasia in Friesian breed

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Ectodermal dysplasia (ED) is a genetic disease characterized by abnormal development of tissues and organs of ectodermal origin, including teeth, hair, nails and sweat glands. There are different forms of ED, the most common of which is caused by mutations in X-linked ectodysplasin gene A (*EDA*). Common features that characterize the affected individuals are hypodontia, sparse hair and absence of sweat glands. In cattle, the gene *EDA* encodes two proteins, EDA1 and EDA2, that differ by the presence or absence of two aminoacids. These two isoforms are members of TFN family. Three mutations that cause ectodermal dysplasia have been described in cattle so far. In our study we screened for mutation two affected calves and some of their close relatives, for a total of 8 animals of Holstein Friesian breed, in order to identify the mutations causing the disease within the *EDA* gene. We identified a new G/A single nucleotide polymorphism (SNP) (GenBank: AJ278907.1, position 30,549 nucleotides) at the 9th base of exon 8 in the *EDA* gene in the two affected calf of Friesian breed. The whole RNA transcribed by the *EDA* gene in affected and healthy animals was sequenced. The observed mutation determines the deletion of the entire exon (131bp). The mutation is located in the exonic splicing enhancers (ESEs) recognized by SRp40 protein. As a consequence, the spliceosome machinery is no longer able to recognize the sequence as exonic and causes exon skipping. The deeply modified RNA causes a severe alteration of the protein and thus the disease. The analysis of this SNP allows the identification of the three possible genotypes (healthy, affected and carrier) and thus highlights not only affected but also carriers animals that can transmit the disease to offspring. This mutation can thus be exploited for a rational and efficient selection of healthy, non carriers animals for breeding.

C-037

Analyses of exercise transcriptome using RNA-Seq

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Horse is a natural athlete and its best economical value is based on the athletic performance and the knowledge of molecular mechanism of exercise-induced stress represent a fundamental prerequisite to obtain better performance and preserve horse welfare. The impact of exercise on the immune response is already established but little is known regarding the early immune responses; it was hypothesized that the physio-pathological condition developing in horses subjected to heavy training (i.e. overtraining syndrome), are based on a derangement of cellular immune regulation. Then, the purpose of this study is to analyze the transcriptome modulation of acute immune response in endurance races, in order to identify candidate genes for such immune system derangement through RNAseq. Solid sequencing was performed on two top level athlete horses using two time points PBMC samples: at rest (t1) and immediately after the race (t2). SOLiD sequencing generated over 182 million 50-base-pairs reads. After quality filtering mRNA-Seq reads were mapped on to the horse genome (Equcab2.0) allowing at least 90% of identity, producing a total of 102 million alignments. Genome coverage distribution was calculated at exonic (57%), intronic (26%) as well as intergenic level (8,7%); 533 genes are down-regulated and 1132 up regulated comparing t2 with t1. All the genes were annotated using the GO annotation available at Ensembl and GO terms enrichment was revealed. Among the most interesting results we found that 12,361 transcripts, mapping on 7208 genes, are potentially novel isoforms as they differ compared to the gene predictions, while 45,901 fragments are annotated as repeats. Finally 74,915 transcripts fall entirely within a reference intron, while 53,213 fragment transcripts are located in intergenic regions. These results highlight the complexity of mammalian transcription during high profile efforts.

C-038**Organic acids evolution in corn silage**

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This research was carried out to study the evolution of organic acids content during ensiling process of whole plant maize in three different conditions: inoculation of *Lactobacillus buchneri*, control and disinfection with sodium hypochlorite. Chopped whole crop maize was ensiled in vacuum polyethylene bags (0.9-1.0 kg per bag) with inoculation of *L. buchneri*, without it and disinfected with sodium hypochlorite. Three silos per treatment were frozen until analysis at -20°C after 0, 12, 24, 48 hour and after 4, 8, 16 and 30 days (72 samples). Immediately after defrosting simultaneous determination of lactic, acetic, propionic, butyric, aconitic, malic, citric and fumaric acids was performed. Data were analyzed by using the GLM procedure of SPSS. Plant organic acids (aconitic, malic, citric and fumaric) lowered their concentration during ensiling process but malic, starting from 8 days after ensiling, kept on increasing until 30 days. Major organic acid found at the end of the process was lactic acid but its faster accumulation rate was recorded on first 48 hours in inoculated and control thesis when pH dropped under value 4. In disinfected samples pH lowered more slowly and its decrease was clear only after 24 hours when also lactic acid began to increase.

After 8 days, in inoculated samples, lactic acid concentration decreased while acetic acid increased. We concluded that major cause of lactic acid production was not bacterial fermentations but enzymatic glycolysis by plant cell enzymes. Treating whole-plant corn with *L. buchneri* resulted in increased concentrations of acetic acid and decreased concentration of lactic acid when compared with untreated corn silage. Sodium hypochlorite did not block only fermentations but retarded also enzymatic glycolysis. Interesting is the rise of malic acid content which was ascribed to a new production during ensiling process despite preservation of initial cell plant content.

C-039**Chemical and nutritional composition of alfalfa at different stages of maturity**

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Alfalfa is one of the most utilized forages in Italy. Numerous studies have shown that yield and quality of forages are both affected by growth stage, forage species, cultivar, climate (e.g. rainfall, temperature) and growing condition. In alfalfa, growth conditions and genetics are probably the main factors responsible for changes in quality, due to the physiological modification of several tissues. This study focused on the quantification of these changes in fibrous and protein fractions and changes in fibre digestibility as a consequence of increased maturity. Eight experimental varieties were harvested every 20 and 30 days in June, July and August 2009. Samples were analysed for their chemical composition; fibrous and protein fraction were determined. Moreover, *in vitro* NDF digestibility was calculated after 24 hour of incubation with rumen fluid. ADL and digestibility data were used for the kD calculation, in order to estimate the rate of digestion per hour. All of these samples were collected by hands from experimental plots, without any loss of leaves during collection and exsiccation. Although observed data were similar between subsequent cuttings, it is possible to note the effects of growing stage in each of the three months. Statistical analysis was performed with the program STATISTICA for a Two way Anova. No differences were observed for the variable Variety, so it was excluded from the model. A significant increased lignification (6.74b% ADL for 20 days, 7.08a% ADL for 30 days of growth), and a less digestible NDF (41.23 a% dNDF 24h for 20 days, 34.29 b% dNDF 24h for 30 days of growth), are together markers of a lower quality forage. In conclusion, no seasonal effects were observed, while maturity still affects samples chemical composition; in alfalfa, collection of the whole plant and genetic improvement should allow producer to obtain good quality forages even during those months when the climatic conditions are not optimal for plants growth.

C-040**Influence of protein fraction content on polyunsaturated fatty acid levels in different forage species**

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Linoleic and linolenic acids in herbage are synthesized in the thylacoids, by the Acetyl-CoA. and their content are related to protein content despite non proteic nitrogen content in fresh herbage is higher. Four species and eight cultivars of *Vicia sativa* L. (VS cv. Jose, JO and Nikian, NI, USA), *Vicia Villosa* Roth (VV cv. Haymaker, HA and Hungjivillosa, HU, USA) *Trifolium incarnatum* L. (TI cv. Viterbo, VI and Contea, CO, USA), and *Trifolium alexandrinum* L. (TAX cv Marmilla, MA and Sacromonte, USA) were compared in a randomized experimental design, to evaluate how PUFA changes respect to different protein fraction, during vegetative and reproductive stages. Protein fractions, linoleic

and linolenic acid contents are strongly influenced ($P < 0.001$) by gender, forage species, cultivars and phenological stages. The results discriminate this relationship and underline that level of protein fraction and PUFA interact between gender, species cultivars and phenological stages. The main PUFA appears to be linolenic acid for all forage species and its best predictor appears true protein (TP) which is positively related with this FA ($y = 0.2501x + 1.6767$; $R^2 = 0.18$ $P < 0.01$). This relationship is more accurate for *Vicia* than *Trifolium* gender, while *Vicia sativa* shows a negative relationship between soluble protein fraction and linolenic acid ($y = -0.0726x + 2.6105$ $R^2 = 0.6986$; $P < 0.01$). Concentrations of TP (14.0 vs 24.2% DM, NS) and linolenic acid (4.65 vs 7.40 mg/g DM $P < 0.001$) were higher during vegetative than reproductive stage. Except for HU, TP is highly correlated ($P < 0.01$) with linolenic acid content but this relationship is strongly influenced by plant phenological stage. Overall gender, species and cultivar effects on PUFA level in herbage are mediated by leaf nitrogen metabolism through a higher FA synthesis efficiency. These results may stimulate future studies aimed at increasing herbage PUFA levels in herbage by nitrogen fertilization.

C-041

Repeatability of *in situ* and *in vitro* digestibility measured at 24 or 48 h of incubation

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In situ and *in vitro* digestibility of ruminant feeds are commonly measured at 48 h of incubation, but shorter incubation times are currently object of investigation for reducing the costs of analysis. The present experiment was aimed to evaluate the correlation and the repeatability of *in situ* and *in vitro* digestibility of feeds measured at 24 or 48 h of incubation. Eleven feeds (seven forages and four concentrates) were analyzed in 4 replications and in 2 separate periods of incubation, using *in situ* (IS), DaisyII (D) and a batch culture (BC) technique, for 24 and 48 h. With IS, in each period the feeds were incubated (1.15 ± 0.005 g) into nylon bags placed in the rumen of two fistulated cows. With D, in each period the feeds (0.25 ± 0.001 g) were incubated into F57 filter bags. Each jar was filled with 0.8 l of rumen fluid and 1.6 L of Menke's medium. With BC, in each period the feeds (0.50 ± 0.001 g) were incubated into individual glass bottles with 25 ml of rumen fluid and 50 ml of Menke's medium. Residuals of fermentation were treated with neutral detergent solution and the true DM digestibility (TDMd, g/kg DM) was computed. *In situ* and *in vitro* TDMd values at 24 and 48 h of incubation were compared by regression, and within technique the coefficient of variation (CV) was used as index of repeatability. The relation-

ships between TDMd values measured at 48 h (y) and 24 h (x) of incubation were: $y = 0.74x + 260$, $R^2 = 0.98$; $y = 0.63x + 353$, $R^2 = 0.95$; $y = 0.66x + 333$, $R^2 = 0.92$ for IS, D and BC, respectively. Except for D, the repeatability of measures at 24 h was acceptable (CV = 4.3, 10.0, and 4.1% for IS, D and BC, respectively) and comparable to that obtained at 48 h (CV = 5.9, 7.2, and 2.9%, in the same order). Results of the present experiment suggest the validity to adopt 24-h incubation time for measuring *in situ* and *in vitro* digestibility of feeds, with advantages in terms of labour efficiency and costs of analysis.

C-042

Effects of soya-bean and rapeseed cake on *in vitro* rumen fermentation and microbial yield

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The aim of the present trial was to evaluate the effect of inclusion of rapeseed cake, obtained from extraction "on farm" (20% EE, 29% CP of DM), on *in vitro* rumen fermentation. Three experimental diets, with a low protein level (L; 11%), were formulated using three different feed sources (soya-bean meal, L_SBM, 3.4% EE; soya-bean seed, L_SBS, 4.6% EE; rapeseed cake L_RSC, 4.5% EE). Two diets, with a high protein level (H, 15%), were also formulated using SBS (H_SBS, 5.8% EE), and RSC (H_RSC, 5.7% EE). Each experimental diet was randomly incubated in 3 replicates into individual vessels. The experiment was carried out using Rusitec system inoculated with fresh rumen fluid collected from 4 cows. In each vessel 20 g/d of diet were incubated in a nylon bag for 48 h. The liquid dilution rate was 27.5 ml/h by continuous infusion of artificial saliva. After 7 days of adaptation, the true OM (TOMd) and NDF (NDFd) degradabilities of diets were calculated. VFA and ammonia N contents were also determined. Samples of total digesta were collected by Rusitec in order to determine microbial nitrogen flow using ¹⁵N as marker. Data were submitted to ANOVA to evaluate the effect of the diet. TOMd and NDFd at 48 h of L_SBM and H_RSC diets were significantly higher ($P < 0.05$) than those of L_SBS and H_SBS diets. Total VFA values were similar in all diets. As expected, ammonia N was greater ($P < 0.05$) in the two diets with high protein level, but no differences were observed between protein sources. Microbial N flow was negatively affected by the lipid content of the diets with maximum value for L_SBM (106 mg/d) and minimum value for H_SBS (59 mg/d). The results of the experiment suggest that "on farm" RSC can be used only as partial replacement of SBM, to avoid negative effects of lipids on microbial growth.

C-043**Development of a calibration curve to estimate dairy cattle dietary particle size with NIRS**Patrizia Carta^{1,2}, Ignazio Ibba², Antonello Cannas¹¹*Dipartimento di Scienze Zootecniche, Università di Sassari, Italy*²*Associazione Regionale Allevatori Sardegna, Italy*
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The use of near infrared reflectance spectroscopy (NIRS) to predict the particle size distribution of total mixed rations (TMR) samples measured with the field equipment Penn State Particle Separator (PSPS) was studied. TMR were collected in 25 dairy cattle farms of Sardinia (Italy) at the beginning and at the end of the feed bunks, for a total of 118 diets and 236 samples. One aliquot of each sample was sieved to determine its particle size distribution by using PSPS. The other was scanned as fresh sample by NIRS. Calibrations were attempted from each spectral origin to predict particle size distribution for >19 mm, from 19 to 8 mm, from 8 to 1.18 mm and <1.18 mm. Validation was performed by using a cross validation of the samples. The PSPS showed the following particle size distribution: Upper fraction (>19.0 mm) SD 8.0%, range 1.3%-50.0% of initial weight; Middle fraction (from 19 to 8 mm) SD 7.3%, range 12.3%-56.5%; Lower fraction (from 8 to 1.18 mm) SD 4.8%, range 24.5%-48.7%; Bottom fraction (<1.18 mm) SD 3.8%, range 9.5%-28%. The standard error of the NIRS prediction (SEP) ranged from 2.310 for the Middle to 1.687 for the Bottom fraction. The SEP for the Upper and Lower fractions were, respectively, 2.219 and 2.133. The highest bias was observed for the Middle fraction (+0.094), whereas the smallest was for the Lower fraction (-0.032). The slope was slightly lower than 1 for the Upper, Middle and Bottom screens (0.989, 0.997 and 0.990, respectively), whereas it was slightly higher than 1 (1.002) for the Lower screen. The peNDF (% of DM) measured by the PSPS and that predicted by NIRS. NIRS also predicted with high accuracy and precision the peNDF (% of DM) measured by the PSPS (as fraction above 1.18 mm). The regression equation of measured on predicted peNDF was: $y = 1.025x - 0.70$, $R^2 = 0.95$, $RSD = 0.760$. In conclusion, NIRS can be used to accurately predict particle size distribution and peNDF of TMR samples.

C-044**Evaluation of gastric degradability of antigenic protein expressed in tobacco seeds**Luciana Rossi¹, Serena Reggi², Simona Vagni¹,
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Plants have been recognized as expression system for the production of edible vaccine because of the possibility of introducing antigenic proteins into their genome. In livestock, transformed plants for the expression of immunogenic proteins, could be administered, orally, in feed to induce mucosal immune response in the gastrointestinal tract. Moreover, edible vaccines for veterinary use could reduce costs of traditional vaccines associated with the production, the cold storage, and parenteral administration. However the most important problem related to the oral delivery route is the potential for antigen degradation in the gastrointestinal tract. For these reasons the aim of this study was the evaluation of the effect of swine gastric fluid on VT2e-B antigenic protein, derived from a strain of Oedema disease *E. coli*, expressed in tobacco seeds by *Agroinfection*. Samples of transgenic tobacco seeds, both milled and whole, were incubated with porcine gastric fluid, at 38°C in Dubnoff Shaker for 1, 2 and 3 hours. After gastric fluid removal, by centrifugation and washing with PBS, samples were homogenized in the presence of protein extraction buffer. Western blot was performed on representative samples of extracted proteins, quantified by Bradford method, using rabbit polyclonal serum. The Vi2e-B specific signal was observed in all samples derived from transgenic tobacco seeds. Nevertheless, from 0 h to 3 h, a progressive reduction of intensity of signal was observed. No significant differences were detected on the reduction of signal intensity between samples derived from whole and milled tobacco seeds.

C-045**Effect of quebracho tannin on fatty acid profile in rumen solid associated bacteria on soya-bean and linseed oils supplemented diets. An *in vitro* study**Sara Minieri, Stefano Rapaccini, Arianna Buccioni,
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Tannins are phenolic compounds which interfere with the biohydrogenation of polyunsaturated fatty acids (PUFA). Aim of this trial was to study the effects of tanniferous extracts of quebracho on the biohydrogenation of linoleic (LA, C18:2 cis9 cis12) and α -linolenic (LNA, C18:3 cis9 cis12 cis15) acids, the most representative fatty acids of soya-bean and linseed oils, respectively. The feed samples used in this *in vitro* study were representative of: two basal diets practically free of tannins, composed of grass hay, soya-bean meal, barley meal, soya-bean or linseed oil (77/5.7/13.8/3.5, on dry matter) used as control (CSO and CLO, respectively); two other diets obtained from supplementation of CSO and CLO with

extracts of quebracho tannins (CSOT and CLOT, 49 g/kg of quebracho tannins). According to the literature it was considered only the fatty acid profile of solid associated bacteria (SAB). The feeds were inoculated with ovine rumen fluid and sampled at 6h, 12h and 18h of fermentation time. At each time of fermentation the samples were collected and fractionated to separate the SAB. Hence, the fatty acid profile was determined by GC. Data were processed by GLM of SAS (1999) using linear model with two factors with interaction: diet and fermentation time. Only one level of probability ($P < 0.05$) was adopted for the significance of differences between means. The data confirmed the attitude of tannins to decrease selectively the rate of biohydrogenation of PUFA, favouring the accumulation of LA and LNA and interfering very little with the hydrogenation of oleic acid (OA, C18:1 cis9). The results suggested that, in order to enrich meat and milk of LNA e LA, it is possible to develop a feeding technique that provides the contemporary supplementation with soya-bean or linseed oils and quebracho tannins extract.

C-046

Enzyme-based sensor for non-invasive monitoring of ruminal parameters: *in vitro* results

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Rumen fermentation of hexoses results in the production of volatile fatty acids (VFA) and release of gaseous carbon dioxide and methane. The partition of glucose C among the different VFA is affected by the diet and has important consequences for ruminant production and the environment. In modern dairy cattle a situation of subacidosis (SARA) is quite accepted due to the need of increasing the energy content of the diet to support high milk production. At present, however, there is no possibility to monitor rumen fermentation parameters at farm level on a regular basis. The availability of a lab-on-chip that would enable the *in vivo* measurement of important rumen fermentation parameters (i.e. pH, lactic acid and VFA concentrations) could be a desirable tool to monitor animal health. In this communication we will present preliminary results concerning the characterization of a chemically functionalized sensor arrays, prospectively mountable onto electronic boluses, able to monitor lactic acid production. The sensor array implements a portable technology based upon the use of back-gated metal-insulator-semiconductor structures where the semiconductor layer is a PEDOT-PSS polymer layer

deposited by spin coating over an oxidized aluminum substrate and modified by grafting lactate dehydrogenase enzymes. The device has been successfully tested for stability under operative conditions by submerging in batches of buffered aqueous solutions and up to 24h in the rumen liquid pool. The results of the test confirmed the usability of the device for monitoring rumen environment, paving the way to actual *in vivo* tests.

Acknowledgement

Project financed by Fondazione Cariplo

C-047

In vitro fermentation characteristics of spineless safflower (*Carthamus tinctorius* L. var. *inermis* Schweinf) grown under different agronomic conditions in a Mediterranean environment

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Safflower has recently aroused interest for forage production in poor areas for resistant to saline condition, moisture stress and capacity in reaching deep-lying water. Its relationship with N fertilization is not well known as well as the adequate N rates. The fermentation characteristics of safflower grown with different N levels was studied with the *in vitro* gas production technique. Four samples of safflower (*Carthamus tinctorius* L. var. *inermis* Schweinf) grown with different N-fertilization (0, 35, 70, 105 kg N ha⁻¹, respectively N0, N1, N2, N3) were incubated in 4 replications, under anaerobic condition at 39°C, with buffalo rumen fluid. The gas produced, recorded with a manual pressure transducer, was fitted to a sigmoid model. Maximum fermentation rate (Rmax) and time at which it occurs (Tmax) were calculated. At 120 h the organic matter degradability (dOM) was determined. Data were analysed to detect the N-fertilization effect. N-fertilization ($P < 0.01$) affected dOM; N2 showed higher value (81.0%) compared to N1 (76.1%) and N3 (75.9%). The gas production tends to increase gradually as level of N-fertilization increase (290, 297, 301 ml/g, for N1, N2 and N3 respectively). Comparing N-treated safflower with the control, N seems favour the fermentation process, decreasing Tmax (6.19 vs 4.49, 4.92 and 3.75 h) and increasing Rmax (12.6 vs 15.6, 13.3 and 16.5 ml/h) for N0, N1, N2 and N3 respectively. In all forages, the fermentation reaches Rmax within 12 h and is exhausted after 48 h. Safflower showed fermentation characteristics comparable with forages commonly fed by ruminants.

C-048**Relationships between end products of fermentation using the gas production technique**

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Feed degradability is frequently measured at 48 h of incubation (t48). However, literature reports that the time (t_{1/2}) at which half of the asymptotic gas production (GP) is more appropriate for microbial studies. At t_{1/2} the products of fermentations (GP; volatile fatty acids, VFA; N-NH₃; N microbial growth, N-MO) should be more correlated than at t48. In the present work the relationships between the various products of fermentation measured at t_{1/2} were studied, using data from a previous trial. The trial evaluated the effects of L-Ascorbic acid (LA), α -tocopheryl (TF) and a phenolic compound (PC) on rumen fermentations of two feeds (meadow hay, MH; corn meal, CM). Feeds were incubated with or without LA, TF and PC in 4 replications. From the GP profiles of a preliminary incubation conducted for 72 h resulted that the t_{1/2} values of MH and CM were approximately 16 and 9 h, respectively. In the experimental incubation each bottle was filled with 0.55 g of feed, 0.01 g of additive and buffered rumen fluid. The GP was measured using an automatic equipment and fermentation was stopped at t_{1/2} and at t48. At the beginning (t₀), at t_{1/2} and at t48 the medium was analysed for VFA, N-NH₃, N in the residual NDF (N-NDF). The N-MO at t_{1/2} and t48 was estimated as: N-feed + (N-NH₃_t₀ - N-NH₃_t) - N-NDF_t. VFA production was used to predict GP (GPp). The products of fermentation at t_{1/2} and at t48 were compared by regression. GP and GPp were better correlated at t_{1/2} (GP = 0.99 × GPp; R² = 0.80) than at t48 (GP = 1.01 × GPp - 39.8; R² = 0.69). Compared to the others additives, PC strongly reduced GP per mg of N-MO. Excluding PC from the regression, GP was strongly related to N-MO at t_{1/2} (GP = 5.75 × N-MO + 36.2; R² = 0.91), but not at t48 (R² = 0.13). These results confirm that in batch culture, N-MO and different products of fermentation should be preferably evaluated at t_{1/2}, the time at which the literature indicates the peak of microbial production is reached.

C-049**Comparison of forage sorghum with grain sorghum and corn silage in diets of lactating cows**

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Aim of the study was to determine the effects of diets based on sorghum forage (*S. bicolor* x *S. Sudangrass*), sorghum grain (*S.*

bicolor) or corn silages on milk yield and digestibility in lactating cows. Three TMR were fed to 6 Italian Friesian cows in a 3x2 Latin square design. Diets had a different silage basis (% on diet DM): 41.5 corn silage (CS); 36.7 sorghum grain (SG) or 28.0 sorghum forage (SF) and a different corn meal level: 16.6, 21.3 and 29.4% for CS, SG and SF diets, respectively. The other ingredients common to all diets were (% on DM): 13.6 dried alfalfa, 1.3 wheat straw and 27.6 concentrate mix. Diets were balanced for the contents (% on DM) of NDF (36%), metabolizable protein (11%) and starch (26%). Urine, faeces and milk produced were measured and sampled daily. Data were statistically analysed by SAS-GLM procedure. Dry matter intake (DMI, kg/d) was lower for SF (18.2) than CS and SG diets (20.0 for both) (P=0.07), probably for the greater particle size of SF diet. Milk yield (kg/d) was lower (P=0.05) for SF (23.6) in comparison with CS diet (25.4) and with no difference vs SG (24.6). Average milk efficiency (1.30 kg milk/kg DMI) was not affected by the diet. Milk fat (%) was numerically higher for SG (4.33), intermediate for SF (4.16) and lower for CS (4.08) (P=0.14). Milk urea N was higher for SF than CS diet (12.9 vs 10.7 mg/dL; P=0.05) with SG being intermediate (11.9 mg/dL) and not different from the other diets. Energy (E) and NDF digestibilities were higher for SF than SG and CS diets (SF: 70.2 and 54.1; SG: 67.7 and 48.6; CS: 68.6 and 51.4 for E and NDF, respectively; P=0.02); a higher rumen retention time of SF diet is the most probable reason for this effect. *In situ* NDF degradability was not different between silages. In conclusion, corn silage appears the most convenient forage, with sorghum grain being intermediate and sorghum forage (likely chopped too long) the worse for feed intake and milk yield.

C-050**Determination of minerals in forages using X ray fluorescence**

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X ray fluorescence (XRF) is a non destructive technique to identify and quantify minerals elements in many different type of materials. The aim of the study was develop to evaluate this analytical technique defining accuracy and repeatability of the method to quantify the most important mineral elements in forages. An energy dispersive instrument (S2 Ranger; Bruker AXS, Milano) potentially capable of measuring element from Na to U was used. About 5g of finely ground (0.5 mm) samples were pressed into a pellet of 40 mm in diameter using a 40Ton laboratory press. The pellet is placed in a shielded chamber where it is irradiated for about 6 minutes with X-Ray at different energy to excite different elements. For this study Na, Mg, P, S, Cl, Ca, K, Mn, Fe and Zn were considered. Twelve certified standard of different vegetable material were used for calibration. The certified values were used as reference for the development of calibra-

tions for the single elements. Samples of corn silage, alfalfa and grass hay used in proficiency ring test in USA (NFTA) were used as independent validation test for Ca, P, K and S. The analysis of validation samples were performed by two operators and repeated over a period of two weeks. Accuracy, repeatability were calculated using Statistica ver. 9. Calibration for the single elements had R^2 of 0.94 or greater with the only exception of Na that was 0.84. Among the elements considered, Na is the lighter one which make it more difficult to quantify for the instrument used in this study. Calibration errors were small for all elements. Among the major ones, calibration error were 0.05, 0.03, 0.17 and 0.01%/DM for Ca, P, K and S, respectively. The repeated predictions of independent samples coming from NFTA, allowed the calculation of accuracy and repeatability of the calibration. Accuracy and repeatability were 0.03 and 0.01%/DM for Ca, 0.02 and 0.01%/DM for P, 0.01 and 0.06%/DM for K, 0.005 and 0.007%/DM for S. XRF is a suitable technique to quantify mineral in forages.

C-051

Diet digestibility by total faecal collection and Acid Insoluble Ash in horses at maintenance fed *S. cerevisiae*

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The aim of the trial was to evaluate the effect of the administration of live yeast to mature horses fed at maintenance by total faecal collection and Acid Insoluble Ash (AIA) methods. Six mares were used in a two-period crossover design with two treatments on the basis of body weight (544 ± 14 kg) and age (15.3 ± 3.9 years): Control (CTR) fed a basal diet, Treatment (LY) fed a basal diet and 2 g/head/d of live yeast (*S. cerevisiae* 4.6×10^{10} CFU/day). The trial consisted of two different periods named period 1 (P1) and period 2 (P2) of 35 days each, including 14 days adaptation period to the diet, 18 days of supplementation with the test material, and 3 days of faecal collection. Each horse was fed 2.50% of body weight at a 70:30 forage:concentrate ratio. Obtained results evidenced a positive effect of live yeast on dry matter (63.94% vs 59.37%; $P=0.03$), and ADF (35.04% vs. 24.30%; $P=0.03$) digestibility, while higher organic matter (66.10% vs 61.60%; $P=0.04$) and NDF (42.20% vs 35.90%; $P=0.04$) digestion rates in LY animals found with the use of AIA as marker were not confirmed by total collection technique, although detected values with both methods were comparable. The administration of *S. cerevisiae* to mature horses resulted in increased digestibility of some constituents of the administered diet that mainly include the fibre fractions such as NDF and ADF.

C-052

Effect of selenium supplementation on plasma glutathione peroxidase and relationship with inflammatory markers in dairy cows

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The relationship between plasma glutathione peroxidase (GPX-3), inflammatory markers and oxidative status in plasma was investigated on 40 Italian Friesian dairy cows. The cows were subdivided in 5 groups: S3 and S5 contained Se yeast *S. cerevisiae* CNCM I-3060 (0.31 and 0.50 mg of total Se/kg DM respectively); N3 and N5 contained sodium selenite (0.31 and 0.50 mg of total Se/kg DM respectively); C as negative control (0.09 mg Se/kg DM). Before the start of the study and after 84, 126, and 140 d, blood samples were collected and plasma analyzed for GPX-3, Se, positive (+APP) and negative (-APP) acute phase proteins, reactive oxygen metabolites (ROM), thiobarbituric acid reactive substances (TBARS) and thiol groups (SHp). Data were processed using mixed procedure for repeated measures, and multiple stepwise regression using GPX-3 as dependent variable. Lower value (n.s.) of GPX-3 was observed in C (263 U/L) vs organic Se (275 and 282 U/L in S3 and S5, respectively) and inorganic Se (305 and 304 U/L in N3 and N5, respectively). The GPX-3 was positively correlated with +APP (haptoglobin: $P<0.001$; ceruloplasmin: $P<0.001$), and negatively with albumin ($P<0.001$). Positive correlations between GPX-3 and ROM ($P<0.001$), and negative between GPX-3 and SHp ($P<0.001$) were observed. The GPX-3 variability was mainly explained (51.2%) by haptoglobin content; conversely the contribute of plasma Se was only 3.7%. A negative relationship between +APP/-APP ratio and SHp as well as between -APP/+APP ratio and TBARS was observed; these relationships were significant only in C, N3 and N5 diets. In conclusion our results highlighted that GPX-3 was greatly affected by inflammatory conditions, and also seem to suggest a lower oxidative stress in relation to the inflammatory conditions in Se yeast cows.

C-053**Nutrigenomics: a high fat diet influences gut gene expression in a mouse model**

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A high fat level in a diet can cause local gut inflammation and leads to metabolic abnormalities, such as obesity and insulin resistance. This study aimed to investigate the effect of a high fat diet in C57BL/6 mice. Twenty-seven mice were divided into five groups. The experiment started at weaning (T0). Five mice were sacrificed at T0 (n=5), while high-fat-fed mice (n=11) and control-fed mice (n=11) were sacrificed after one (T1, n=5 for each treatment) and two weeks (T2, n=6 for each treatment) of such dietary regimens. Even though neither blood nor histological analysis gave evidence of a clear inflammatory state in the caecum after 1 or 2 weeks of treatment, we used 90K Combimatrix microarray technology to check if the expression level of genes was changed. A total of 29,435 probes in triplicates were considered, representing all the available mouse genes in database. After hybridization and scanning, the Cy5 signal intensities were analyzed using limma package from Bioconductor. Only 7 genes were detected differentially expressed between control and high fat diet mice, out of the whole transcriptome (q-value ≤ 0.05), with a range of log fold change between -2.06 and 1.22. Among statistically significant genes 4 were successfully validated by real time PCR. In conclusion, genes differentially expressed after two weeks of treatment all shared an involvement in the regulatory pathway of the circadian clock system, which was recently shown to affect lipid metabolism and inflammatory processes.

C-054**Effect of whole linseed addition on the expression of some lipid metabolism genes in the adipose tissue of Italian Simmental and Holstein young bulls**

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The objective of this trial was to determine the effect of breed and whole linseed addition on the expression of some genes involved in the lipid metabolism in subcutaneous adipose tissue of young bulls. Thirty-two young bulls were assigned to four groups following a factorial design 2 breeds (Italian Simmental, IS and Italian Holstein, IH) x 2 diets (containing 8% of DM of ground whole linseed, WL and without linseed, CON). At slaughter the 8th rib sample joint was taken and dissected with the aim to predict the carcass composition and samples of subcutaneous adipose tissue (head tail) were collected, from which RNA was extracted. The mRNA abundances of stearoyl-CoA desaturase (SCD), fatty acid synthetase (FAS), lipoprotein lipase (LPL), leptin (LEP), PPAR γ (Peroxisome proliferator-activated receptors) were detected by real-time PCR. Changes in gene expression were calculated using the delta-delta Ct method. Two-way ANOVA was used to determine the effects of diets and breeds. In the LEP mRNA expression analysis, the estimated carcass fat was used as a covariate. Results showed that the inclusion of linseed, rich in C18:3 n-3, decreased the expression of SCD gene (P<0.05), LPL gene (P<0.05), and tended to decrease the expression of FAS gene (P=0.056) without affecting the LEP and PPAR γ mRNA abundance in the adipose tissue of bulls. No breed effect was found on the gene expression, also the interaction diet x breed was considered, but it never reached a level of significance (P>0.05). In our trial the linseed supplementation reduces the gene expression of key lipogenic enzymes in subcutaneous adipose tissue of young bulls by a mechanism that does not involve the PPAR γ regulation.

C-055**Specie-specific probiotic supplement in veal calves diet: effects on zootechnical and microbial parameters in standard rearing conditions**

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The aim of the study was to evaluate the effects of the administration of a specie-specific probiotic supplement to veal calves on performance and microbial parameters in standard rearing conditions (SRC). Ninety-six male Friesian veal calves (49.31 \pm 1.38kg) were divided in two homogeneous groups of 48 animals each from the arrival in the farm and fed either a basal diet (C) or a basal diet plus 1.8x10¹⁰ CFU/head/day of a probiotic supplement containing

B. coagulans, *L. animalis* and *L. paracasei* spp. *paracasei* in a 35:30:35 ratio (T) for a total of 180 days. Performance and microbial parameters were analyzed by a MIXED procedure of SAS (2006), while dressing percentage was evaluated by a GLM (SAS, 2006). Average daily gain (1.19 vs 1.21 kg/d respectively for T and C) and dressing percentage (55.69 vs 55.75%) were not different among groups. pH at 45 min and 48 hours *post mortem* were similar for C and T; no significant differences were detected between T and C groups for faecal microbiological parameters in whole experimental period, but *Lactobacilli* content increased on month 4 in T calves (8.87 Log₁₀/g vs 8.35 Log₁₀/g, P≤0.10) when antibiotic treatments were not performed; also faecal score was found to be better in the same period in T than C. The General Health Score was higher in T than C after the second month and it showed, on month 6, a significant difference. In SRC the administration of a species-specific probiotic supplement did not influence growth performance, but positive changes of treated animals health must be further investigated.

C-056

Dietary supplementation of mannanoligosaccharides in nutritionally stressed piglets: effects on gut health

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The aim of the trial was to evaluate the effect of the administration of mannanoligosaccharides (MOS) on growth performance, villi height and crypts depth of gastrointestinal tracts, intestinal inflammatory responsive parameters and microbial population in faeces and caecum of piglets fed a low digestible diet. Forty-eight weaned piglets (6.72±0.32 kg of BW, 24 d of age) were used in a 35-d experiment and randomly allotted to 2 dietary treatments: basal diet (Control) and basal diet + 0.2% MOS. Growth performance were recorded weekly, faecal samples were collected at 0, 14 and 35 d. At the end of trial, 10 piglets from each group were slaughtered and intestinal samples were collected. Data were analysed by a General Linear Model (GLM) procedure of SAS. BW, ADG, ADFI were not influenced by MOS supplementation; FCR was lower in treated animals in the last 2 weeks. Mean faecal score was improved in MOS piglets. At the end of trial treated piglets had higher anaerobic, aerobic bacteria and *Lactobacilli* faecal count. No difference was detected among groups for Coliforms, while lower *Clostridia* occurred on day 14 in MOS piglets. Intestinal villi height in the duodenum was higher in MOS than Control. MOS supplementation also led to significant increase of NO production in ileal mucosa. Results indicate that MOS supplementation slightly improved feed efficiency and intestinal morphometry of piglets fed low digestible diet.

C-057

Effect of fasting and refeeding on the expression of the complex of genes involved in the control of the gastric ghrelin orexigenic signal of pigs

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Knowledge on orexigenic signals in the pig stomach is poor. The gastric release of the active ghrelin (octanoyl-ghrelin) is under the complex control of 3 genes: prepro-ghrelin, proprotein convertase (PC1/3), for the posttranslational cleavage, ghrelin O-acyltransferase (GOAT), for acylation of pro-ghrelin. Twelve pigs (12.0 kg LW) adapted to a base diet for 1 week, were then divided in 3 groups of 4 pigs and slaughtered 3 days later. Each group had an individual feeding schedule: a. Control (C), fed twice a day for the whole trial, b. Refeeding (R), fasted on d 8 and then re-fed after 24 hours until the slaughter; c. Fasting (F), fasted on d 9 until the slaughter. Per each pig, total RNA was isolated from fundic and pyloric mucosa (Takara Fast Pure™ kit protocol) and reverse transcribed (ImProm-II Reverse Transcription System). Specific primers were designed on the sequence of pig Pre/Pro Ghrelin, PC 1/3, and GOAT (GenBank), by Primer 3. The quantification was performed in a LightCycler instrument, using Takara SYBR Premix Ex Taq II. In the fundic mucosa the prepro-ghrelin gene expression tended to be increased with the refeeding (P=0.09), fasting and refeeding increased PC1/3 (P<0.01), and GOAT was not affected. In the pylorus mucosa the GOAT mRNA was increased by refeeding (P<0.05), while prepro-ghrelin and PC1/3 were not affected. Compared with the other two genes, the expression of PC1/3 is higher in pyloric mucosa. The pool of genes involved in the secretion of active ghrelin are active both in fundic and pyloric mucosa, while in general in other species only fundic mucosa has been evaluated; fasting and refeeding can affect the expression of genes that control octoanoyl-ghrelin production; a higher PC1/3 mRNA presence in pyloric mucosa is consistent with the observation that PC1/3 is required also for the processing of gastrin.

C-058**Mammary gland proteoma in sheep**

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In this study we aimed to identify the proteins that describe the biochemical pathway involved in sheep lactation, through the detection of the differentially expressed proteins in the mammary gland at the three most peculiar lactation stages: beginning, peak and end lactation. Moreover, to identify the pathway responsible of the basic processes of milk production, the study was carried out both on three ewes of a specialized dairy breed (Sarda) and three ewes of a breed that has never been selected for milk production (Gentile). The animals were reared in the same experimental farm and fed with the same diet. One ewe for each breed was slaughtered at each of the following lactation stages: early lactation (one week after lambing); mid lactation (weaning of the lamb); late lactation (one week before the dry-off). Two-dimensional gel electrophoresis (2-DE) was applied to the proteins extracted by mammary tissues collected from each ewe. Each sample was carried out in triplicate. Gels were stained with colloidal Coomassie. To analyse the changes over time in the mammary proteins, the spots with a difference of ≥ 2 -fold change of photodensity between two different lactation stages in both breeds were selected. Only the spots showing the same trend along the lactation in both breeds were submitted to mass spectrometry and identification was performed by searching NCBI mammal database. The proteins were classified based on KEGG database. Two major groups of proteins were detected: the most abundant group includes the proteins of metabolism and explains the variation in the request of energy of the mammary gland for milk synthesis and secretion along the lactation, with maximum photodensity value at lactation peak. The second group includes the proteins involved in cell apoptosis, and confirms the occurrence of this process as far as the lactation progresses. Our results show that the proteomic approach is appropriate to provide information on the biochemical pathways that produce specific phenotypes.

C-059**Feeding system effect on chemical and fatty acid composition of Amiata ass's milk**

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Ass's milk has recently been indicated as a nutraceutical food gaining attention for its interesting profile of fatty acids and for the DHA, EPA and ARA levels. Aim of the trial was to compare the effect of feeding system on chemical and fatty acids composition of Amiata ass's milk. For the trial 10 asses, from 90 to 146 days of lactation, were used and assigned into two groups fed with different diets. One group received a basic forage diet (FO) with access to pasture while the other group was stabled and fed with forage and concentrate (CO) according the NRC requirements for lactating donkeys. Every 15 days the donkeys were milked and the trial lasted for 60 days. The chemical composition and the fatty acids profile were investigated. Data were submitted to ANCOVA using the model: $y_{ij} = \mu + \alpha_i + \beta \cdot x_{ij} + e_{ij}$; where α_i = effect of diet, x_{ij} = days of lactation. Significant differences ($P < 0.01$) were found for fat and protein content with the lower values in pastured animals (fat: 0.28% vs 0.44%; proteins: 1.63% vs 2.02%). The fatty acids profile was slightly different in the two groups. In stabled animals was found a significant higher value ($P < 0.01$) in total PUFA (17.27% vs 14.78%), linoleic acid (9.56% vs 7.27%) and EPA (0.19% vs 0.13%). Data showed that the feeding system influenced the chemical and fatty acids composition of the milk. Donkey milk used for infant and human diseases should have a standardized composition before being commercialized. Further studies should be carried out to find the best diet to improve the lipid fraction composition.

C-060**Influence of days in milk and parity on milk and plasma IgA in dairy cows**

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Milk is a source of antimicrobial factors that protect newborn from infection and provide biodefense against infectious agents in the mammary gland. In particular, IgA limits bacterial translocation in the suckling by enhancing gut mucosal barrier function and promotes toxin neutralization, stimulates agglutination, preventing bacterial colonization in the alveoli.

In the light of this, the aim of the study was to evaluate the effect of day in milk and parity on IgA level in colostrum, milk and plasma of dairy cows. Seven primiparous (PR) and 7 multiparous (MU) Friesian cows were studied for 3 months after calving. On day 0, 1, 3, 7, 30, 60 and 90 of lactation, milk yield was recorded, colostrum, milk and blood samples were taken. Colostrum and milk samples were obtained mixing morning and evening milkings and then tested for total bacterial count. Skimmed colostrum/milk and plasma were tested for IgA by ELISA. The

effect of day in milk and parity on colostrum, milk and plasma IgA was tested by ANOVA. Maximum milk production was observed on day 30 for MU cows (43.3 L/d) and on day 60 for PR ones (40.5 L/d). On day 0, 60 and 90 total bacterial count was lower in milk from PR than MU cows ($P=0.06$). Concerning milk and plasma IgA, in both groups the highest levels were found at calving. The day after parturition IgA concentrations dramatically decreased in both plasma and colostrum, remaining at low levels for the rest of the study period. On calving day plasma IgA was higher ($P<0.01$) in PR than MU cows (137 vs 46.4 $\mu\text{g/mL}$), whereas colostrum IgA was lower (640 vs 782 $\mu\text{g/mL}$; $P<0.01$). Milk IgA was positively correlated to plasma IgA ($P<0.01$) during the first month of lactation, and negatively related to bacterial count ($P<0.05$) after the first month. This study gives a description of the pattern of IgA in plasma, colostrum and milk of dairy cows during the first months of lactation and its relation to milk bacterial count.

C-061

Different approaches to modelling the temporal evolution of milk yield in small ruminants

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Analysis of lactation curves in small ruminants often presents difficulties due to the incidence of environmental effects and the absence of selection. In order to compare both pre-adjusted data and methods to fit test day (TD), 7,638 Sarda dairy goat lactations were first analyzed by using both the Multivariate Factor Analysis and Principal Components Analysis. These techniques were able to extract two new variables (F_n and PC_n): F_1 and F_2 were factors related to peak and persistency, respectively; PC_1 and PC_2 were correlated with general level of production and persistency, respectively. The new variables were analyzed with the following linear model:

F_n or $PC_n = \text{Parity} + \text{Month of kidding} + \text{Altitude of flock} + \text{Flock} + e$. In addition, a sub-set of 1,584 lactations with the first TD recorded at <30 DIM were analyzed with: Wood, Cappio-Borlino, third order Legendre polynomials and splines (linear, quadratic and cubic) functions, fitted on both averaged TD (Av) and TD smoothed by using the LOWESS regression (Sm). $F_1 + F_2$ and $PC_1 + PC_2$ explained 65% and 77% of total TD (co)variance, respectively. The new variables were significantly affected by all classification factors ($P<0.001$). F_1 and PC_1 scores were the lowest in first parity and increased with parity order. F_1 scores increased from October to February, whereas PC_1 values decreased. The production level in early (F_1) and whole lactation (PC_1), and persistency (F_2 and PC_2) differed markedly among altitude locations of flocks. All fitted functions showed the same goodness of fit. However, the regular patterns of lactation curves were recon-

structed better by models with a small number of parameters than by polynomial functions. Finally, all models showed better fitting performances using Sm data ($0.97 < R^2 < 0.74$) than using Av data ($0.79 < R^2 < 0.53$). The results of this study suggest that phenotypic data analysis can improve using the LOWESS regression and multivariate techniques. The scores of F_n and PC_n could be useful to discriminate and select animals with different value of lactation traits.

C-062

Effect of plant compounds on blood cell count of dairy sheep under ACTH challenge

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The effect on blood cell count (BCC) of four plant bioactive compound dietary supplementation has been evaluated in ewes under Tetracosactrin (ACTH) challenge. Thirty-six Sarda sheep were randomly assigned to six groups: CTR (no ACTH and supplementation), ACT (ACTH and no supplementation), LD (ACTH and 50 g/head/day of *Larix decidua*), ECHI (ACTH and 3 mg/kg live weight/day of *Echinacea angustifolia*), ANDRO (ACTH and 1 mg/kg live weight/day of *Andrographis paniculata*) and POLI (ACTH and 3 mg/kg live weight/day of Polinacea™). After 22 days of adaptation to the experimental diets, all the groups, except CTR, were injected twice a day with 0.5 mg of ACTH for 3 consecutive days. Blood was sampled before (T_0) and after 3 (T_3) and 51 (T_{51}) hours from the first injection and used for the BCC employing an automatic analyzer. Data computation was performed using the repeated measure statement of the SPSS. Hematocrit and red blood cell count didn't vary during the trial. Haemoglobin (Hb) levels at T_{51} decreased for all the experimental groups ($P=0.000$). Contrast analysis showed at T_3 a different trend of Hb concentrations between ACT group and the other groups ($P<0.01$). White blood cell count increased with time of sampling ($P=0.000$), an effect of group was evident on this parameter ($P<0.01$), indeed the mean values of PO, EA and LD were higher than for the other groups. Neutrophils increased with time of sampling ($P=0.000$) but contrast analysis showed a different trend for CTR group compared to the other groups at T_{51} ($P<0.01$), keeping a value similar to that of T_0 . On the contrary, lymphocytes decreased in all the experimental groups ($P=0.000$) except for CTR group, as confirmed by contrast analysis ($P<0.01$). Monocytes decreased after the first ACTH injection, except for CTR group in which the percentage significantly increased ($P<0.05$). Tetracosactrin injection showed important effects on BCC regulation and bioactive compounds were able to modulate this changes.

C-063

The effect of the foaling season on lactation length and milk production of jennets

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The effects of the foaling season and the lactation phase on the milk productive variables were investigated on 34 pluriparous jennets fed with hay, concentrate and pasture. From the 21st post-foaling day, the jennets were manually milked without the foal, one time a day, after 5 hours from the foal' separation. Every 3 weeks, the individual milk yields were recorded and individual milk samples were collected and analyzed. The foaling season influenced the jennets' length of lactation ($P < 0.001$). In general, the jennets foaled in spring and in summer showed longer lactations, in relation to the climatic conditions in which the animals evolved the different lactation stages. However, the total milk produced in the lactation did not show differences, because of the jennets foaled in summer, with the lowest daily milk yield, showed the longest lactations. The foaling season influenced the fat ($P \leq 0.05$), the protein ($P \leq 0.001$) and the lactose contents ($P \leq 0.001$), as the somatic cell score (SCS) ($P \leq 0.001$). The daily milk yield of the jennets foaled in spring, in summer and in winter showed an evident peak in the first lactation phases, differently of the jennets foaled in autumn. After the aforementioned times, the daily milk yield decreased until the end of the lactation. The fat trend would seem influenced by the forage availability. In agreement with the normal lactation tendency, a higher protein content were found in the first lactation phases, decreasing during the lactation. The lactose content increased appreciably until the 150th day and after decreased. If one milking in a day is adopted, it would seem convenient to milk the jennets until 8-9 months post foaling.

C-064

Evaluation of the milk fatty acid profile from core and membrane of milk fat globules during lactation

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Milk triglycerides form approximately 98% of the total fat content of milk. They make up mostly the core of the fat globule but the membrane also contributes to the total fat content of milk. The aim of this study was to assess the milk fatty acid profile from the core and membrane of milk fat globules during lactation.

The trial was carried out on seven Massese ewes kept indoors at 20 days post partum. The animals were homogeneous in terms of parity and feed. Individual milk samples from the morning milking were collected at 30, 45, 60, 90 and 120 days post partum and were analyzed for milk fatty acids from whole milk and the core and membrane of milk fat globules. Core and membrane was separated according to the method described by Patton and Huston (1986). Each analysis was carried out in duplicate and the results were elaborated using a model for repeated measurements. The results showed that fatty acid composition of the milk fat globule membrane compared with the composition of the core had more saturated fatty acids, particularly C16:0 (+21.5%) and C18:0 (+67.6%) and polyunsaturated (+48.7%). On the other hand, the core of milk fat globules had a higher content of mono-unsaturated (+11.0%) and short chain fatty acids (+84.4%).

C-065
Quality of beef meat aged in moderate cooling room

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Meat production is a high energy requiring process. So, due to global request of sustainable producing techniques, it is often exposed to strong criticisms. Moreover, meat's price may result high and access to meat uneasy. It is worthwhile to set up alternative strategies particularly with regard to ageing and preservation. The aim of our study was the evaluation of quality of beef meat aged in moderate refrigeration conditions (8°C vs 2°C) five days long. NaCl and CaCl₂ have been added to meat aged at mild temperature (8°C) in order to contain bacterial spoilage and to speed ageing up respectively. After the first period of ageing all samples were kept 5 days more in order to evaluate shelf life under three different thermal conditions (2,4,8°C).

Six Friesian bullocks were slaughtered. Shoulders were separated and split into two groups. One group was aged at a 2°C temperature (F) and the other, after spraying by a mixture containing NaCl 4M and CaCl₂ 400 mM, at 8°C (C). Microbiological samples were done at days 1st and 5th and oxidation (TBA) and other meat quality traits were evaluated at days 1st, 5th and 10th. All data were processed by GLM procedure of SAS using a factorial model with interaction with thermic treatment, muscle, and storing temperature and time as factors. From a microbiological point of view, both groups F and C remained below the level of acceptability imposed by law, at day 5th. Some important meat quality traits (e.g., Warner Blatzer Shear Force on cooked meat - WBS_c), were positively affected by C treatment (confirmed by sensory test). Oxidation (TBA) and colour (L*, a*, b*) were not different between groups F and C at day 5th. Use of active-films for shelf life extension is needed.

	F	C
WBS _c (kg)	6.24±0.85 ^a	5.06±0.37 ^b
Tenderness (panel)	3.81±1.01 ^b	5.02±0.73 ^a

Different letters means $P < 0.01$. Values registered 5 days after slaughtering.

C-066
Effects of ventilation system on beef production and meat quality

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Heat stress may be avoided with use of cooling systems such as ventilation which in livestock housing allows maintaining a comfortable animal environment. The aim of this study was to verify the influence of a ventilation system on beef production and meat quality. The study was carried out on 10 Charolais young bulls raised in two pens: with (YES) and without (NO) ventilation system. Continuous measurements of air temperature and relative humidity were conducted from July 21 till September 13. Thermo Hygrometric Index (THI) was calculated following Kelly and Bond formula (1971). At slaughter carcass weights and SEU-ROP evaluation were performed. The day after, wholesale ribs (11th-13th) were dissected. After 7 and 14 days of ageing: pH, water losses, WBS on cooked and raw meat and colour parameters (L*, a*, b*) were performed. One way variance analysis (GLM) was used to evaluate the significance of differences. When the fan was off, both distribution and average values of THI in the two boxes were not significantly different, while THI was lower in the ventilated box when the fan was on (YES 77; NO 77.5). Cooling system proved to be effective in reducing THI. However, no significant difference on slaughter performances, wholesale rib dissection and physical parameters was found, except for the redness index (YES: 6.97, NO: 10.02) and Chrome values (YES: 12.31; NO: 16.97) after 7d of ageing time. However a multivariate supervised model (PLSDA) could discriminate between the two groups on the basis of the observed (independent) variables. The variables that contributed more to the discriminant analysis were fat %, meat %, pH, WBS, L* and a*.

C-067
Effect of alternative proteins in the diet on physical-sensory beef quality

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Twenty Friesian young bulls were used to determine the effect of different protein sources on meat quality. Bulls (10 for group) were fed with field bean or chickpea; the diet contained 0.97 UFV/kg DM and CP 12.3%. The animals were slaughtered at about 550 kg BW. After 7 days of chilling treatment, samples of *Longissimus thoracis* muscle were removed. The samples were used for physical quality analysis at 7 and 14 days: drip loss, cooking loss and hardness (WBS); chemical analysis: total (TC) and insoluble collagen (IC); sensory parameters were evaluated with the use of a structured continuous scale of values between 0 and 10 (0=absence of sensation; 10=greatest intensity of sensation);

flavour intensity, butter flavour, lemon flavour, beef broth flavour, off flavour, initial tenderness (IT), masticability (M), final fibrous texture (FFT) and initial juiciness were analysed. The physical-chemical data were subjected to one-way variance analysis, while the sensory data to three-way (assessors; reply and samples) variance analysis with GLM procedures. All data were processed with CORR procedures. Among the physical-chemical parameters, only hardness was affected by the diet where chickpea gave higher tenderness in meat (6.14 kg vs 7.95 kg at 7 d and 5.01 kg vs 6.06 kg at 14 d). About sensory attributes, only initial tenderness (5.07 vs 6.20) and masticability (5.1 vs 5.8) resulted higher in chickpea diet. This difference is due to the negative correlation between sensory attribute and WBS (-0.61 at IT and WBS 14 d; -0.69 at M and WBS 14 d); the WBS was significantly correlated with FFT (0.57 at 14 d) too. Collagen was correlated with butter flavour (TC 0.47; IC 0.52). The investigated protein sources significantly affected only tenderness, both in WBS and sensory attributes.

C-068

Effect of crossbreeding with Belgian Blue sires on carcass weight and market value of beef bulls and heifers

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Aim of this study was to estimate the effect of four breed crosses on carcass weight (CW, kg) and market value (MV, Euro/carcass) of beef bulls and heifers obtained from the mating of Belgian Blue sires with cows from two dairy (Brown Swiss and Holstein Friesian) and two dual purpose (Simmental and Rendena) breeds. Data on MV and CW from 1,530 males and 1,718 females were recorded from 2006 to 2009 by the Breeders Federation of Trento province. Heifers and bulls originated from one and 8 fattening farms, respectively. After editing procedure, analysis of variance was carried out using the GLM procedure of SAS and two different models that accounted for the fixed effects of breed cross, fattening farm (only for bulls) and the date of slaughtering. The coefficients of determination of the models for CW were 0.55 and 0.42 for bulls and heifers, respectively, and for MV were 0.64 and 0.69, respectively. All effects were highly significant ($P < 0.001$) in explaining the variability of the studied traits. The highest least squares means (\pm SE) for CW and MV were found for bulls and heifers from Simmental dams (369 ± 3.0 and 278 ± 1.2 kg, and $1,668 \pm 13.9$ and $1,541 \pm 7.0$ Euro/carcass, respectively), whereas animals from Holstein Friesian dams exhibited the lowest values of CW (356 ± 3.1 and 272 ± 1.3 kg, respectively) and MV ($1,599 \pm 14.7$ and $1,502 \pm 7.8$ Euro/carcass, respectively). Results suggest that good economic revenues can be achieved from crossbred animals, particularly when Belgian Blue sires are used on dual purpose cows.

C-069

Image analysis using Computer Vision System for meat colour evaluation

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This research aimed to implement an alternative technique of image analysis based on Computer Vision System (CVS) for meat colour evaluation and compare it with the colorimeter. The study was carried out on three species: cattle (*Longissimus dorsi*, *Semimembranosus* and *Semitendinosus*), pig (*Longissimus dorsi*) and chicken (*Pectoralis major*) using 15 samples per muscle. Meat colorimetric characteristics (L^* , a^* , b^*) were measured using both a Minolta Colorimeter CR-400 and a CVS. The latter relied on a camera CANON EOS 450D at high-resolution for image acquisition. Lighting was achieved with four fluorescent lamps with a colour temperature of 6500 °K. The camera was connected to a PC NEC LCD monitor with sRGB gamut. Colour management was performed by the Adobe Photoshop CS3 software. Differences between colorimeter and CVS measurements in terms of L^* , a^* and b^* were located by the Student's t-test. The results showed significant differences between the values obtained using the two equipments. Beef lightness measured with the colorimeter was always higher than L^* evaluated by CVS ($P < 0.05$). An opposite trend was observed in pig ($P < 0.001$) and in chicken ($P < 0.001$) meat. The a^* , b^* , C^* and h values gathered through CVS were always higher than those obtained using the colorimeter ($P < 0.001$). A similarity test between the actual samples and the colours gathered from the two equipments was conducted using a 15 member trained panel. The images and colours reproduced by the CVS and derived from the transformation of L^* , a^* and b^* in RGB were more similar to the colour of the samples than the colour derived from the transformation in RGB of L^* , a^* and b^* obtained by the colorimeter ($P < 0.001$). These results suggest that the colorimeter is not suitable for meat colour evaluation due to the translucency and lack of homogeneity of the matrix. CVS, on the contrary, may be considered a valid technique to reproduce the real colour of meat.

C-070

Proteolysis changes in Podolian beef during ageing time: a proteomic study

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Proteome analysis was used to assess the influence of ageing time on beef Podolian tenderness. The study was carried out with 8 Podolian young bulls slaughtered at 24 months of age; *Longissimus dorsi* (LD), *Semitendinosus* (ST), and *Psoas major* (PM) muscles were removed from each half carcass 24 h post mortem. Each muscle was divided into two sections resulting in four samples for each animal: one was analyzed immediately and three after ageing at 2°C in vacuum packaging for 7, 14 and 21 days, respectively. Warner Bratzler share force (WBS), myofibrillar fragmentation index (MFI) and changes in myofibrillar and sarcoplasmatic proteins were estimated. The extracted myofibrillar and sarcoplasmatic fractions were analyzed by SDS-PAGE (8-18%) and two dimensional gel electrophoresis (2DE). All data were subjected to an analysis of variance using the GLM procedure with ageing time as the main effect. During the ageing, WBS significantly decreased ($P<0.001$) in LD and PM muscles, MFI increased ($P<0.001$) in all muscles, SDS-PAGE showed a change in intensity and number of proteins bands for the myofibrillar and sarcoplasmatic fractions in all muscles. In particular, bands corresponding to myosin light chain 1 (MLC1) increased ($P<0.01$) between 7 and 21 days post-mortem, while sarcoplasmatic proteins as glyceraldehydes-3-phosphate dehydrogenase (GAPDH) and creatine kinase (CK) bands decreased ($P<0.05$) in intensity. 2DE analysis highlighted that ageing time influenced the composition of the myofibrillar fraction resulting in higher proteolysis, revealed by an increase in the number of the spots (from 88 at 159; $P<0.01$). The proteolysis has involved especially the MLC that are implicated in the acto-myosin complex. The sarcoplasmatic fractions showed that no effect of ageing was observed in the number of the spot. The proteomics approach proved that ageing causes changes on proteolysis and tenderness of Podolian meat.

C-071

Effects of grazing on animals and on pastures in organic farms in Central Italy

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EU organic Regulation states that organic animal farming should be based mainly on grazing in order to assure animal welfare and a proper exploitation of pastures. To investigate the effects of grazing on animals and on pastures, an interdisciplinary research was performed in an organic beef farm in Tuscany with a pasture area of 46 ha. For 2 years, DM production, botanical composition and carrying capacity data from 2 different type of farm pastures, old (O) and new (N), grazed for about 180 days year⁻¹, were collected. In order to evaluate effects of grazing on

animals, 8 Limousine young bulls grazed from March to June in the N type pastures and after were fattened indoors according to the EU organic Regulation (P), while 8 were reared always indoors (I). Performances and zoometric indices were recorded, and variable fattening costs were calculated. All data were compared by ANOVA. Average DM production of O pastures (5.82 t ha⁻¹) resulted significantly lower than N (7.74 t ha⁻¹). Grasses dominated the pastures (average 58%) due to their high suitability to grazing. Legumes were poorly represented, with higher value in N pasture (17%) than in O (13%). Utilisation rate was very high (average 86%) demonstrating a good exploitation of pastures by animals. Real stocking rate was on average 105% of carrying capacity (evaluated only in the O pastures) indicating that a light overgrazing is present in the studied pastures with effects on legumes presence that are more affected by heavy grazing. Data collected on animals showed a slaughtering body weight at 19 months not significantly different between the two experimental groups (P 631 vs I 609 kg), but grazing influenced in a positive way both the chest height (P 41.2 vs I 37.9 cm) and the cost for 1 kg of daily gain (P 0,79 vs I 1,11 €). Results showed that age of pasture can affect in a remarkable way the quantitative and qualitative pastoral parameters and indicated that organic young bulls may be conveniently raised on the pasture.

C-072

Organic meat from Italian Holstein young bulls

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The increased interest on organic meat promoted the dairy breed calves fattening, to produce high quality and low cost organic meat. To investigate on meat quality and optimal slaughtering age, 10 Italian Holstein organic calves were reared in natural pasture and after fattened indoors according to the EU organic Regulations. Animals were slaughtered at 14, 15 and 16 months of age. Behavioural observations were carried out on pasture and indoors; blood samples were collected before and after grazing period; *in vivo* and *post mortem* performances and zoometric indices were measured; physical and chemical meat analyses, fatty acid profile and panel test were performed; variable costs were calculated. Behavioural data were analyzed by chi-square and others data by ANOVA. Meat quality results were compared with bibliographic data regarding others beef breeds.

Mg, Albumin and Total Protein significantly increased and Globulin decreased. Behavioural observations showed more social activities after grazing period. Different slaughtering age didn't show significant influence on performances (live weight

kg 390, 460, 477; dressing percentage % 52; 52; 51). 15 months young bulls showed, in fatty acid profile, higher Aterogenic Index (0.55B; 0.69A; 0.52B) with values comparable to organic Limousine and Chianina meat. Trombogenic Index (0.70; 0.96; 0.96) was lower than organic Limousine meat and similar to Piemontese. Polyunsaturated n-3 content (% 3.45; 2.39; 2.98) resulted higher than the Chianina and Calvana meat. Slaughtering age didn't influence panel test, but meat resulted sweeter (2-3% points), less acid (2-3% points), tenderer (8-10 points) and more chewing-able (10-15 point) than that of organic Limousine. Variable costs for 1 kg of daily gain (average of the three groups € 0.67) resulted lower than in organic Limousine (average € 0.79). In conclusion, 16 months, when the performances are still high and meat quality excellent, seems the optimal slaughtering age for Italian Holstein organic young bulls.

C-073

Performance and nutritive value of Creole goat kid's meat, reared under low input systems

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A study was conducted to investigate the performance and nutritive value of goat kid's meat during two seasons (summer 2007 and winter 2009). Four groups of ten Creole male goat kids grazed without supplementation in a caducifolious forest located in Guerrero state, Mexico. Animal performance, carcass yield, protein, ash, moisture, total fat, fatty acid profile, polyphenol, caffeic acid and catechine evaluations were performed (*Longissimus dorsi* muscle). Lower weight gains during summer period compared to winter rates were observed. Final weight was not different among groups ($P>0.05$). As well, carcass yield and protein were not affected by the seasonality ($P<0.05$). Higher intramuscular fat during winter was detected ($P<0.05$). SFA and MUFA concentrations were larger in winter and produced a healthier $\omega-6/\omega-3$ ratio ($P<0.05$). Polyphenol, caffeic acid and catechine content on meat coming from summer grazing were detected. Goats reared under low input conditions whether on summer or winter may contribute to sustain local demands of meat supplies.

C-074

"Agnello di Sardegna" PGI: lamb meat production under different livestock systems

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The effects of different livestock systems on slaughter age (SA), slaughter weight (SW), average daily gain (ADG), slaughter parameters, and colour coordinates (CIE) of PGI "Agnello di Sardegna" heavy lamb were studied. Twenty-one lambs, progeny by Ile de France sires and Sarda ewes, after suckling period of 50 days, were divided into the following groups homogeneous for sex and twin birth: group Stall ($n=7$) weaned and stall-fed with 0.765 kg head⁻¹ day⁻¹ of alfa-alfa hay and 0.5 kg head⁻¹ day⁻¹ of commercial concentrate; group Grass ($n=7$) weaned and fed only at pasture (*L. italicum*) for 24 hours a day; group Milk, ($n=7$) suckled by their mothers. The mothers grazed for 7 hours per day and received as supplements alfa-alfa hay (0.5 kg head⁻¹ day⁻¹) and commercial concentrate (0.5 kg head⁻¹ day⁻¹). All data were tested with GLM procedure using livestock system as fixed effect. The tested livestock systems significantly affected SA, ADG, carcass weight, chilled dressing percentage (CD), whereas SW, pH and CIE (L^* ; a^* ; b^*) showed no difference between the three carcass groups. Milk group showed the lowest SA (77.4±2.9 days *vs* 92.3±2.9 and 92.1±2.9 days for Stall and Grass, respectively, $P<0.01$), the best ADG (0.292±0.01 *vs* 0.229±0.01 and 0.231±0.01 kg head⁻¹ day⁻¹, for Stall and Grass, respectively, $P<0.005$) and CD (61.18±2.0 *vs* 53.33±2.0 and 55.07±2.0% for Stall and Grass, respectively, $P<0.05$). The measured meat pH at 24 hours *post-mortem* ranged between 5.72±0.1 and 5.87±0.1. The average colour coordinates of *Longissimus dorsi* and *Transversus abdominis* muscles, recorded at 24 hours post-mortem, were 35.5±2.7 L^* , 17.1±1.5 a^* , 3.8±1.0 b^* in *L. dorsi* and 42.9±2.8 L^* , 17.3±2.2 a^* , 6.6±1.8 b^* in *T. abdominis*. The livestock systems affect heavy lamb performance with best results in Milk system. As already found in our previous study no difference was observed in Stall and in Grass farming systems results.

C-075

Effect of natural and artificial milk on long chain fatty acid of meat from Sarda suckling lambs

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A study was carried out to evaluate the effect of ewe's milk versus artificial rearing on the fatty acid (FA) profile of suckling

lamb meat with emphasis on long-chain FA. Twenty-four Sarda suckling lambs were divided into two groups (12 lambs each) at birth and reared exclusively on maternal milk (M group) or on artificial milk (R group). Lambs were slaughtered at 28 days of age. After 24 h of refrigeration at 4°C, the tight muscles were dissected from each right half-carcass. FA profile of milk and meat was determined by gas-chromatography. Data were analyzed with one-way ANOVA using milk source as the main factor. Significant differences between maternal milk and milk replacer were observed for almost all FA. Milk replacer had a higher content of C18:2 n6, PUFA n6 and total PUFA and a lower content of t11 C18:1, c9,t11 CLA ($P<0.01$) and SFA ($P<0.10$) than maternal milk. The proportion of PUFA n3 did not differ significantly between the two feeds due to the similar content of C18:3 n3. FA profile of meat was significantly influenced by milk source. Meat from M lambs had a higher proportion of t11 C18:1 (2.34 vs 0.00%) and c9,t11 CLA (1.63 vs 0.08%) than that of R lambs ($P<0.01$). The amount of C18:2 n6, C20:4 n6 and PUFA n6 were higher in R lambs compared to M lambs ($P<0.01$). On the other hand the content of C18:3 n3 was higher ($P<0.01$) in M lambs (1.26 vs 0.82%). The contents of EPA, DPA, DHA and PUFA n3 were higher in M lambs compared to R lambs even if the differences did not reach the level of significance. The proportion of PUFA was higher ($P<0.01$) and that of SFA lower ($P<0.01$) in meat from the R group, resulting in a higher ($P<0.01$) PUFA/SFA ratio (0.68 vs 0.24) while the value of the n6/n3 ratio was lower ($P<0.01$) in meat from the M lambs (2.08 vs 7.97). In conclusion, a milk-feeding regime based on artificial milk reduced the nutritional value of lamb meat compared to a natural rearing system, lowering the level PUFA n3 and c9,t11 CLA and increasing the n6/n3 ratio.

Acknowledgement

The research was funded by University of Sassari (F.A.R. grant).

C-076

Fatty acid oxidation of meat from lambs fed diets supplemented with extruded linseed and olive cake

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The use of linseed in the diet increases alfa-linolenic acid (LNA), n-3 PUFA and conjugated linoleic acid (CLA) content in the meat fat. However, feeding animal with linseed potentially increases the susceptibility of the meat fat to oxidation, favoring off-flavor pro-

duction. The use of antioxidants in the diet may be a solution to prevent these problems. At this aim, in the present study, olive cake as source of antioxidant substances was used as alternative to Vitamin E supplementation in lamb feeding. The experiment was conducted on 32 Appenninica lambs, equally assigned to 4 experimental groups: control (C), linseed (L), olive cake (O) and olive cake + linseed (OL). Samples of *longissimus dorsi* muscle were stored at 4°C and analysis for antioxidant content and lipid oxidation were repeated at 0, 4, 7 and 11 days during the storage period. Antioxidant (tocopherols and retinol) content was analyzed by HPLC. Lipid oxidation was determined by 2-thiobarbituric acid reactive substances (TBARS) analysis and cholesterol oxidation product analysis. The use of olive cake in the diet increased the meat antioxidant content (+52%), particularly tocopherols level, which remains higher after 11 days of storage. The higher antioxidant level in animals fed with O and OL diet resulted in a greater protection of meat against lipid oxidation. At time 0 the amount of TBARS in lamb meat was not different across diets. After 11 days of storage at 4°C the amount of TBARS significantly increased only in meat of lambs fed C and L diet. These results encourage the use of olive cake in diet supplemented with linseed, to reduce the lipid meat oxidation phenomena related to the higher level of PUFA.

C-077

Effect of the length of pasture finishing on triglyceride and phospholipid fatty acid composition of intramuscular fat from lamb meat

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Aim of the trial was to study the effect of pasture finishing on fatty acid (FA) composition of intramuscular lamb meat fat, by analyzing total lipids (TL), phospholipids (PL) and triacylglycerols (TG) composition. After weaning (60 days long), 40 Italian Merinizzata lambs were equally allotted into 4 groups according to 4 different feeding regimen: P, 84 days pasture; S, 84 days with a diet composed by concentrate (65% barley, 35% chickpea) and grass hay; PF34, 50 days pasture then 34 days diet S; PF14, 70 days pasture then 14 days diet S. TL content of meat from S-group lambs resulted 2 times higher than that from other groups (2.4, 1.5, 1.3 and 1.3 g/100g of meat, in S, PF34, PF14 and P, respectively, $P<0.05$). The proportion between TG and PL varied according to meat fatness, being higher in C samples and lower in S, PF34, PF14 samples. Feeding regimen strongly affected FA composition of TL, especially in the case of FA which are prefer-

entially esterified in TG. The content of PUFA n3 increased with the increase of the length of the grazing period (0.5, 0.5, 1.3 and 2.0 g/100g of FA in S, PF34, PF14 and P, respectively, $P < 0.0001$). The content of PUFA n6 increased as the length of pasture finishing decreased as a consequence of the increase of concentrate supply (10.1, 11.7, 11.7 and 12.5 g/100g of FA in P, PF14, PF34 and S, respectively, $P = 0.015$). Both trends were confirmed with a larger extent in the PL fraction, because PUFA n3 and n6 are preferentially esterified in PL. Feeding regimen dramatically affected n6/n3 ratio (21.7 in S vs 5.2 in P, $P < 0.0001$), but also SFA content (39.8% in S vs 41.6% in P, $P < 0.001$), PUFA content (19.8% in S vs 21.4% in P, $P < 0.0001$) and the PUFA/SFA ratio (0.5 in S vs 0.6 in P, $P < 0.0001$), whereas any effect was revealed on MUFA content.

C-078

Influence of extruded linseed dietary supplementation on lipid profile and sensory lamb meat quality

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Nutritionists recommend an increase in the intake of n-3 long-chain fatty acids (FA) as well as an increase in the ratio of polyunsaturated fatty acids (PUFA) to saturated FA and/or in the C18:3 n-3 to C18:2 n-6 ratio in dietary lipids. The effects of extruded linseed (EL) content in concentrates on growth performance and FA composition and sensory properties of *Semimembranosus* muscle (SM) of 81 Sarda lambs (initial live weight: Kg 15.3±0.8) were studied in a 2x3 design: males vs. females and EL content (0%, L-0, 10%, L-10, 20%, L-20). The experimental period was 30 days. Sensory quality of SM from L-0 and L-20 lambs, either plain or seasoned, was assessed through consumer test. No effects of EL contents in the diet on growth performances were observed (final live weight: kg 17.9±0.9). Data were analysed using a one-way ANOVA model. Carcass quality traits were not affected by the feeding regime. FA composition of SM was not significantly influenced by diet, except for the proportion of C17, C17:1 and C18:2n-6 trans which decreased with the EL content in the concentrate. However, both n-6 and n-3 PUFA tended to increase, and n-6/n-3 ratio to decrease, with EL supplementation. Consumer testing did not show any difference in overall acceptability and sensory traits in plain meat. A higher score for extraneous flavours was assigned by panellists to the L-20 seasoned samples. In conclusion, the daily intake of EL evaluated in this experiment did not appear to affect growth perform-

ance, lipid profile and sensory properties of lamb meat. Further studies are required to assess the effects of using greater amounts of concentrates in the diet of intensively-reared lambs.

C-079

Lamb meat oxidative stability as affected by the length of concentrate-based finishing diet

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This study was designed to assess the effect of the duration of feeding lambs with concentrates in stall after a period of pasture feeding on meat oxidative stability. Thirty-eight Italian Merino lambs were divided into 4 groups at 60 days of age. Over 89 days, 10 lambs (S group) were fed concentrates in stall, while 9 animals (P group) grazed herbage at pasture. Ten lambs (P-S37 group) and 9 lambs (P-S14 group) initially grazed together with animals in the P group, but were housed in stall and fed as the S group for 37 and 14 days before slaughtering, respectively. Lipid oxidation was measured as TBARS (mg of malonaldehyde / kg of meat) on slices of raw muscle *Longissimus dorsi* and on cooked minced muscle over 8 and 2 days of storage at 4°C, respectively. After 8 days of storage, lower TBARS values were found in raw muscle from lambs in the P group (0.70) compared to the S, P-S14 and P-S37 groups (2.32, 2.13 and 3.09, respectively; $P < 0.0005$), while meat from lambs in the P-S37 group displayed higher TBARS values compared to S and P-S14 groups ($P < 0.05$). After 2 days of storage, TBARS values in cooked meat from S, P-S14 and P-S37 groups (2.48, 2.03 and 2.41, respectively) did not differ, but were higher compared to the P group (1.47; $P < 0.05$). These results suggest that possible modifications of the balance between pro-oxidant and antioxidant components in muscle may be responsible for a different lipid stability between meat from lambs fed exclusively herbage at pasture and meat from animals fed exclusively concentrates or finished on concentrates after a period of pasture feeding.

C-080
Does aging of turkey males affect the cryosurvival of spermatozoa?

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It is well known that males aging had a negative impact on sperm quality and consequent fertility. This is relevant in turkey industry where males are raised for semen production when they are from 29 to 60 wk aged. Then, despite the dependence of this industry on artificial insemination, only fresh semen can be used because sperm cryopreservation still not lead to good fertility results. However, little is known about the aging effect on cryosurvival of turkey spermatozoa. This work aimed at evaluating the effect of turkey male's age on the *in vitro* quality of semen post-thaw. 5 pools of semen (9-12 ejaculates/pool) were collected from Hybrid Large White toms when males were 32, 44 and 56 wk old. An aliquot from each pool was taken for the analysis on fresh semen, the remaining was four-fold extended, cooled, added with 8% of dimethylacetamide as cryoprotectant and frozen by dropping 80 μ L of semen directly in liquid nitrogen to form frozen pellets. The samples were quickly thawed at 75°C for few seconds. Sperm mobility (Accudenz swim-down test), viability (SyBr-PI staining) and osmotic-resistance (HOS-test) were examined on fresh and post-thawed spermatozoa. Results showed that in fresh semen a low sperm concentration, mobility and osmotic-resistance were found in semen collected from 56 wk old turkey compared to that collected from 32 wk old toms ($P < 0.05$). The freezing/thawing process caused a significant reduction of semen quality at all ages ($P < 0.05$), however post-thaw sperm viability was significantly higher ($P < 0.05$) when males were 32 and 44 wk old compared to that of 56 wk old toms (34.92 ± 2.97 and 42.31 ± 4.24 vs 23.71 ± 8.79 , respectively) and osmotic resistance was higher ($P < 0.05$) when males were 44 wk old (33.05 ± 4.80) than 56 wk old (18.46 ± 5.48). These results show that the age of turkey males influences not only the quality of fresh semen, but also the cryosurvival of spermatozoa.

C-081
Effects of egg yolk/albumen ratio on hatched chick body composition and broiler meat traits in dual-purpose genotypes by computer tomography

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Computer tomography (CT) was used to select eggs with different

yolk/albumen ratios (Y/A) and to check selection method effectiveness in improving hatched chick body composition and broiler meat traits. CT selection was used on two genotypes: a dual-purpose genotype (Tetra-H; Bálolna TETRA Kft.; genotype LL) and a genetic line originated from Golden Plymouth and selected for growth rate (genotype EE). CT analysis involved 3500 eggs per genotype. Eggs with the lowest (10%, n=350), average (10%, n=350), and highest (10%, n=350) Y/A ratios were incubated. After hatching, 15 chicks per sex and genotype (180 in total) were weighed, sexed suppressed, and body proximate composition was determined. Those remaining were marked and reared with ad libitum feeding to slaughter at 11 weeks of age. Slaughter weight was 2.6 and 3.5 kg for LL and EE. Fifteen thighs per group underwent physicochemical analysis. ANOVA tested effects of genotype, Y/A ratio, and their interactions. EE hatched chicks were heavier (36.6 vs 35.5 g; $P < 0.01$) and richer in moisture (76.8 vs 75.5% ; $P < 0.001$) than LL chicks, while LL hatched chicks had higher protein (16.1 vs 16.8% ; $P < 0.001$) and ether extract (3.90 vs 4.37% ; $P < 0.001$). Low Y/A ratio eggs produced heavier chicks with higher moisture and protein content but lower ether extract. 11-week-old LL broiler meat was higher in moisture than EE meat (74.9 vs 73.8% ; $P < 0.001$) but ether extract was higher in the latter (4.10 vs 5.12% ; $P < 0.001$) due to higher growth rate and precocity. Genotype affected all the rheological traits. As expected, EE thighs had significantly higher meat/bone ratio, fat content, and tibia strength ($P < 0.001$). The low Y/A ratio compared to the other 2 ratios increased thigh meat content and thus thawing loss ($P < 0.05$). The results show the Y/A ratio useful in selecting chicks before hatching and that lower Y/A ratio increased carcass meatiness with no change in proximate composition.

C-082
Effect of free range production system on chicken carcass and meat quality traits

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There is a growing consumer interest towards poultry products coming from unconventional housing systems with outdoor access. A study was conducted to characterize carcass traits and chemico-physical properties of chickens labelled free-range (according to the EC Directive 1538/91) if compared with conventional ones. Free range female (FR-F) and male (FR-M) chickens were separately raised for 56 and 70 d, respectively, in order to obtain currently marketed product categories. They belonged to medium growing Isa strain and had continuous daytime access to open-air area from 28 d to slaughter age and indoors stocking density did not exceed 27.5 kg/sqm. Conventional female (C-F) and male (C-M) chickens belonged to a fast growing hybrid (Ross 708) and were separately raised for 39 and 50 d, respectively, under intensive conditions in a poultry house under controlled environmental conditions at a stocking density of 30-32

kg/m². Females (FR-F and C-F) and male (FR-M and C-M) birds were slaughtered into 2 separate sessions. After slaughtering, 20 carcasses for each group (FR-F, 1.2 kg; FR-M, 1.9 kg; C-F, 1.2 kg; C-M, 2.3 kg) were randomly selected for evaluating carcass cut-up yields and fifteen of them were used to assess quality properties of both breast and leg meat (skin and meat colour, ultimate pH, drip and cooking losses, AK-shear force). C birds had dramatic higher carcass and breast meat yield, whereas FR had higher wing and leg yields ($P < 0.001$). Both meat and skin of breast and leg coming from FR birds were lighter and less red and more yellow ($P < 0.001$). Even if no difference were found in ultimate pH, FR birds exhibited higher water-holding capacity (lower drip and cooking losses) in both breast and leg meat ($P < 0.01$). Finally, although shear force did not differ in breast meat, leg from FR birds were tougher ($P < 0.001$). Overall these data indicate that noticeable quality trait differences exist between free range and conventional labelled poultry products.

C-083

Effect of free range production system on safety and microbiological parameters of poultry carcasses

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A study was conducted to compare the microbiological properties of chickens labelled free-range (according to the EC Directive 1538/91) if compared with conventional ones. Free range chickens belonging to medium growing Isa strain were raised for 70 d with continuous day-time access to open-air area from 28 d to slaughter age and an indoors stocking density not exceeding 27.5 kg/sqm. Conventional chickens belonged to a fast growing hybrid (Ross 708) and were raised for 50 d under intensive conditions. During slaughtering, 20 birds per each group before and after plucking, after evisceration and refrigeration (i.e. 5 birds per each step) were randomly collected and used for the quantitative evaluation (i.e. Log CFU/carcass) of total aerobic bacteria, *Enterobacteriaceae* and *Campylobacter*. Moreover, the detection of *Salmonella* was performed on 5 refrigerated carcasses per group as well as on their intestines (also *Campylobacter* was assessed) and neck skin of 10 additional birds. All microbiological determinations were performed using ISO based methods. Moreover *Salmonella* was further detected and *Campylobacter* quantified using traditional and RT-PCR, respectively. The total aerobic and *Campylobacter* counts before (i.e. 7.88 vs 8.39 and 6.05 vs 7.04 Log CFU/carcass) and after (i.e. 7.29 vs 7.75 and 5.09 vs 6.31 Log CFU/carcass) plucking were significantly lower in free range than conventional birds. The *Enterobacteriaceae* count showed the same trend but only after plucking (i.e. 6.42 vs 7.03 Log CFU/carcass). In both conventional and free range systems, positive *Salmonella* neck skin samples collected before refrigeration were obtained (i.e. 2/5 vs 3/5). However, all carcasses tested after refrigeration but one, belonging to conventional birds, turned out as negative.

C-084

Effect of protease supplementation on nitrogen excretion in broiler chicken farming

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The aim of this study was to evaluate the effects of protease supplementation to low and high protein diets on the reduction of nitrogen excretion of heavy broiler chickens. A total of 1320 males Ross 708 one day-old chickens were equally divided into 4 groups according to the feeding program and housed in 24 pens of 6 m² each (6 replicates per group). Two groups received high protein feeds (from 217 to 163 g/kg feed), supplemented (HPP) or not (HP) with protease (15,000 U/kg feed); the other 2 groups received low protein feeds (200 to 144 g/kg feed), supplemented (LPP) or not (LP) with protease (15,000 U/kg feed). Birds were reared on litter till 50 days and slaughtered at a liveweight of 3.5 kg. The protease promoted a higher nitrogen body retention only in LPP group (30.6 vs 29.3 g/kg liveweight respectively for LPP and LP; $P < 0.05$) as well as a lower nitrogen excreted/ingested ratio (47.9 vs 49.3%; $P < 0.01$). Overall HP and HPP groups showed a higher nitrogen excretion in comparison with LP and LPP birds (31.8 and 31.8 vs 28.9 and 28.2 g/kg liveweight respectively; $P < 0.01$). The incidence of foot pad dermatitis was lower in LP and LPP groups regardless of the protease addition due to the lower litter nitrogen content (72 vs 78 g/bird) rather than the litter moisture which was similar among groups. The protein level and the enzyme supplementation did not affect the production performance with the exception of feed efficiency which was lower in birds receiving high protein diets in comparison with those receiving low protein diets (1.98 and 1.98 vs 2.04 and 2.04 respectively for HP, HPP, LP and LPP; $P < 0.05$). Carcass yields and meat quality too were not affected by the protein level and enzyme supplementation. In conclusion, low protein diets fed to heavy broiler chickens reduced the nitrogen excretion while the protease supplementation improved the nitrogen retention but only when added to low protein diets.

C-085

Sperm DNA fragmentation after cryopreservation in meat type chicken breeders

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The comet assay or single cell gel electrophoresis (SCGE) has become a recognized method for detecting DNA damage in a variety of verte-

brate cell types, including spermatozoa. The comet assay is used in several domestic animal species to investigate the potential damage induced by common semen handling practices, such as cryopreservation. The aim of the present study was to study DNA fragmentation in chicken spermatozoa after processing for cryopreservation, in F15 Hubbard strain selected for meat production. Semen collected from 7 males was diluted in modified Lake's pre-freezing extender, frozen in pellets and thawed in water bath at 60°C. Comet assay and viability (SYBR14-PI) were performed on fresh semen soon after collection and dilution, and after thawing. The proportion of spermatozoa with damaged DNA significantly increased from 5.7% (fresh semen) to 28.3% (frozen-thawed semen) during cryopreservation. The proportion of viable spermatozoa significantly decreased from 77.5% to 20.4% in fresh and frozen-thawed semen respectively. Sperm DNA damage was much lower compared to the decrease in viable sperm following cryopreservation, therefore, damages to nuclear DNA are not considered as a major cause of cryoinjury in chicken semen. However, we propose that comet assay is suggested as a useful tool in multiparametric sperm assessment to investigate sperm sensibility to cryopreservation in chicken.

C-086

The effect of cold storage and carcass weight on the shelf life and quality characteristics of whole and deboned broiler breast meat

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The study was conducted to investigate the effect of cold storage period, carcass weight and product form on fresh broiler bacteriology and meat quality parameters. A total of 500 one-day old broiler birds were raised to a market age before slaughtering. Carcasses were graded into two categories; light weight (1100±50g) and heavy weight (1400±50g). After 4 h of post-chilling aging, 256 carcasses were randomly selected to represent the two categories. Each category of 128 carcasses was randomly distributed into 2 groups of 64 carcasses. One group was stored as whole carcass while the other group was stored as cut-part deboned breast meat at 4°C for 1, 3, 5 and 7 days of cold storage (16 samples/storage day for whole carcasses and deboned breasts). Data were analyzed as a factorial arrangement in a completely randomized design using GLM procedure of SAS. Post-chilling temperature, pH, cooking loss, water holding capacity and shear force were significantly affected by product form and storage period. Water holding capacity and shear force were also affected by carcass weight ($P<0.001$). L^* , a^* , b^* , chroma and hue values were significantly affected by storage period. The L^* value was only affected by product form and carcass weight ($P<0.01$). Crude protein and ether extract were significantly affected by carcass weight and storage period, while ash was only affected by carcass weight. Aerobic plate count

(APC), psychrotrophic count (PTC), proteolytic count (PLC), lipolytic count (LLC) and coliform count (CC) were significantly increased with storage time. The shelf life of fresh broiler chicken meat ranged between 3 to 5 days. In conclusion, carcass weight did not affect overall meat quality while meat started to deteriorate after 5 days of cold storage.

C-087

Adaptability of different poultry genotypes to organic system

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According to (EC) Reg. 889/2008 the choice of breeds or strains for organic system, shall take into account animals capacity to adapt to local conditions, vitality and resistance to disease and a wide biological diversity should be encouraged. Thus the aim of present study was to evaluate adaptability to organic system of eight chicken genotypes (100 birds each) different for growing rate: very slow (Robusta maculata, R; Livorno, L; Ancona, A); slow (Gaina, G; Cornish x Livorno, CL; Cornish x Ancona, CA); medium (Kabir, K; Naked neck, N). Statistical analysis of data was done using a linear model considering the genotype effect. The N, G and K genotypes showed the lower number of behaviours (7, 7 and 8, respectively), while for A and CA was 13 and 15 ($P<0.05$); L, R and CL performed 100% of behaviours very far from observers at pasture. These results confirmed the great kinetic activities of local breed and their crossbreed respect to commercial ones. In fact N, K and G chickens performed more than 50% of activities in resting and about 20% in feeding at feeders, while others genotypes in kinetic activities at pasture ($P<0.05$). Respect tonic immobility, the higher values were reached by N and K chickens that showed food and sterne pads which may explain their low motor activity; in fact when birds get heavier, it hurts them to keep standing up and/or moving, so they spent much of their time laying on excrement-filled litter. This was also confirmed by the percentage of culled bird at slaughter (3%) for both N and K genotypes. N chickens reached a satisfactory slaughter weight at 81 (2.50 kg), K (2.38 kg) at 90 and G (2.14 kg) at 100 days, whereas the crossbreeds (2.26 and 2.45 kg for CA and CL, respectively) at 120 and the pure breeds (1.9 and 1.7 kg for A and L, respectively) over 150 days. Mortality showed an opposite trend respect to productivity. In conclusion, on the basis of the above mentioned EC Regulation, the better compromise among environmental adaptability, biodiversity and productivity is given by the two crossbreeds.

C-088**The trichological analysis in the study of local sheep breeds**

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In general, an anatomic-morphometric approach using dichotomous keys is the first step of a preliminary study. This work has as objective the study of this approach to analyze the hair of local sheep breeds to determine if it can highlight the possible differences between races. In the case of unidentified population in the genus *Ovis* the use of dichotomous keys based on the examination of trichological sections can mislead because of mistakes by the technician. Hairs from the local sheep breeds, 4 Venetian local sheep (Alpagota, Brogna, Foza and Lamon) and 1 other sheep breed (Appenninica) were examined. The hairs were extracted from the interscapular zone and stored in paper bags. Six hairs from the under hair area (referred to as GH2) per 4 individuals per breed were randomly chosen. Hairs have been mounted on a glass-slide according to the Teerink method (1991), slightly modified by us, with a transparent enamel cast for the cuticle and entire sample for the medulla. Optical observations were performed at 200x and digital Leica software pictures have been made for each area of Shield (SD) and Shaft (SF). Areas (μm^2) and perimeter (μm) of the sections, of whole cells, the scales and the empty intracellular parts were measured in five areas both for SF or SD by means of ImageJ for microscopy software. The analysis performed by the R version 2.12 software has shown by ANOVA analysis, both globally and after exclusion of the largest areas of the hair sections, that the medians of the examined areas (cuticle and medulla) were statistically different. In particular, emerged as the Appenninica breed is different from other breeds ($P < 0.01$). Results showed that this easy and low expensive method can be useful for characterization of local sheep breeds with potential benefit for mammal populations.

C-089**Use of locally weighted scatterplot smoothing (LOWESS) regression to study genome signatures in Piedmontese and Italian Brown cattle breeds**

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Selection is the major force affecting local levels of genetic variation in a species. Genomics and the use of dense marker maps offer new opportunities for a detailed understanding of genetic diversity across the animal genome. Over the last fifty years, cat-

tle breeds have been subjected to intense artificial selection. Consequently, regions controlling traits of economic importance are expected to exhibit selection signatures. The fixation index (F_{st}) is an estimate of population differentiation, based on genetic polymorphism data, calculated using the relationship between inbreeding and heterozygosity. The aim of this study is to develop a new statistical approach to detect signature selection of the genome among cattle breeds with different production types. F_{st} and observed heterozygosity (H_{et}) were calculated for 43,766 SNPs marker loci randomly distributed across the genome in 749 Italian Brown and 364 Piedmontese bulls. Then, both F_{st} and H_{et} were fitted with a Locally Weighted Scatterplot Smoothing (LOWESS) regression to yield chromosomal smoothed patterns. LOWESS predicted trends of both F_{st} and H_{et} were compared. The statistical significance of F_{st} values was assessed by using a control chart. The LOWESS technique was efficient in removing noise from the raw data and was able to highlight selection signatures for H_{et} and F_{st} . Examples are the peaks detected for BTA6 in the regions harbouring ABCG2 and casein clusters and for BTA2 in the region where the miostatin locus is located.

C-090**Genetic diversity of five Italian sheep breeds**

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This study, included in a wider project on "Perception of the quality and traceability of the sheep products", is aimed to study the genetic diversity of 5 Italian sheep breeds, namely Appenninica (AP), Pomarancina (PO), Istriana (IS), Comisana (CO) and Merinizzata Italiana (ME). Diversity was assessed using 12 microsatellite markers. Blood samples from 233 individuals belonging to investigated populations were genotyped. Observed (H_o) and expected (H_e) heterozygosity were estimated. Nei and Reynolds genetic distances among breeds were computed, a neighbour-joining consensus tree was constructed and the tree robustness was evaluated by bootstrapping over loci (10,000 replications). Furthermore allele sharing distances among individuals were determined. Population structure and degree of admixture was assessed using the STRUCTURE 2.2 software for $1 \leq k \leq 15$. The results showed that a heterozygote deficiency is present for each breed, with IS showing the largest difference (0.078) and ME the smallest (0.005). Nei and Reynolds distances gave similar results and three clusters could be identified: one formed by PO, AP and ME whereas IS and CO clustered separately. Radiation tree from inter-individual distances shows defined clusters only for IS and CO animals while AP, PO and ME animals are mixed. The analysis of STRUCTURE output showed that the

best combination of ΔK and $\ln Pr(X|K)$ was for $k=5$ which is the real number of population used. In conclusion the investigated breeds show a good genetic diversity except for IS breed in which the risk of inbreeding is still high (both in terms of low heterozygosity and heterozygote deficiency). The use of only 12 microsatellites allows to discriminate all the investigated breeds quite well, with better result for IS and CO that have distant geographical origins, while it was more difficult to discriminate animals of PO, AP and ME breeds because of the common recent history.

C-091

Genetic diversity of seven Italian sheep breeds as inferred by genome-wide SNP analysis

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High-throughput SNP genotyping is more and more becoming a cost-effective analytical tool also in the sheep species. Here we present the preliminary results of a joint collaborative project aiming at characterizing genetic variability and genetic structure of the most representative Italian sheep breeds. Samples from seven breeds (Altamura, Appenninica, Comisana, Leccese, Pecora Nera di Arbus, Sarda and Sopravissana) were genotyped using the Illumina OvineSNP50 BeadChip. After pruning of SNPs according to minor allele frequency ($MAF < 0.01$; 336 loci), genotype missingness ($Geno > 0.1$; 231 loci) and linkage disequilibrium ($r^2 > 0.5$; 3338 loci), 45129 loci were left available for subsequent analyses. Overall observed heterozygosity was 0.362 ± 0.117 , with the lowest value observed in Leccese and the highest in Comisana. Gene diversity was 0.383 ± 0.118 for the total sample, with the lowest value observed in Pecora Nera di Arbus and the highest in Sopravissana. The IBS distance multi-dimensional scaling plot highlighted a clear separation of the two Sardinian breeds from the others along the first component, probably reflecting geographic isolation. The second component separated the Appenninica breed from the others, likely as a consequence of more than thirty years of selection toward the meat production. A similar but more pronounced breed differentiation was highlighted by the NeighborNet network obtained from the IBS distance matrix and by the Admixture analysis (at $K=7$, each cluster mostly corresponded to a different breed sample). These results confirm the power of the genome-wide SNP analysis to

resolve breed relationships also among closely related breeds, thus providing an optimal tool for fine dissection of sheep genetic structure.

C-092

A phylogenetic overview of the three Italian Merino-derived breeds from a mitochondrial DNA standpoint

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The Italian Merino-derived breeds represent a significant animal resource in terms of genetic diversity and socio-economic relevance. The aim of this study is to provide a mitochondrial DNA (mtDNA) overview of Sopravissana, Gentile di Puglia and Merinizzata Italiana, thus improving the awareness relative to the genetic diversity and population structure of these breeds, which has been previously studied through a microsatellite-based analysis. This study takes into account also the Appenninica, as hypothetical female ancestor of these Merino-derived breeds. A total of 120 blood samples (30 for each breed) were collected from unrelated individuals of different flocks (Sopravissana: 14; Gentile di Puglia: 11; Merinizzata: 5; Appenninica: 10). The analysis was performed on 809 base pairs (nps 15452-16260, relative to the NCBI Reference Sequence NC_001941.1) of the mtDNA control region and all sequences have been analyzed and phylogenetically compared. Intriguingly, only one haplotype (mutation motif: 15721-15800-15820-15858-15871-15912-15923-15931-15978-16128), was shared between Gentile di Puglia and Sopravissana, in spite of the assumed common origin of the three Merino-derived breeds. Based on these data, it is likely that different ancestors could account for the present mitochondrial gene pool of these Italian stocks. In order to deeper investigate the geographic origin of such ancestors it will be necessary to extend this investigation to other Italian and non-Italian breeds. The resulting findings will certainly represent a starting point for the safeguard and promotion of important autochthonous genetic resources in Italy.

C-093**Pagliarola population or Appenninica breed: a microsatellite discrimination**

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A flock supposed to belong to the Pagliarola (PA) sheep population was found in the L'Aquila province. According to many authors PA sheep mainly live in the centre and south Italy and has to be considered one of the local breeds; PA is thought to be one of the ancient ancestors of the current Appenninica (AP) sheep. Aim of this study was to verify if the considered flock could belong to PA, or it might only be a group of crossbred animals derived by the breeds most popular in the same area (AP; Gentile di Puglia, GP; and Merinizzata Italiana, MI). DNA was extracted from blood of 40 PA individuals. Thirty STR loci, included in the FAO panel, were amplified and the data statistically processed together with those coming from the reference breeds (30 animals from AP, GP and MI, respectively). The PA sample is characterised by a rather high genetic variability (high number of alleles, calculated by microsatellite toolkit), and it generally respects the Hardy-Weinberg equilibrium, as confirmed by a Fis value not statistically different from zero (0.036, estimated by FSTAT software). The inter-individual DAS distances tree (estimated by neighbour-joining algorithm, by the software Population) showed a defined cluster for PA, as all the animals strongly grouped together, confirmed by the 18 private alleles. According to the Factorial Correspondence Analysis, PA parted from AP and was located near GP and MI. According to the Fst genetic distance estimates, the most separated breeds were PA and AP (0.1). In conclusion, it is reasonable to suppose that the studied flock is not a sub-population of the current AP and represents a defined and unique genetic type that could be assigned to the ancient Pagliarola.

Acknowledgement

This research was supported by "Ente Parco Nazionale del Gran Sasso e Monti della Laga"

C-094**Genetic structure and assignment test in five sheep breeds reared in Sicily using microsatellites**

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A total of 331 individuals from five sheep breeds reared in Sicily

(Barbaresca (BAR, n=57), Comisana (COM, n=65), Pinzirita (PIN, n=75), Sarda (SAR, n=64), and Valle del Belice (VdB, n=70)) were analysed to investigate genetic structure and perform an assignment test. A panel of 20 microsatellite markers was used, and a total of 259 alleles were observed. The polymorphic information content (PIC) all over loci was equal to 0.757, showing that the microsatellites panel used was highly informative. Estimates of observed heterozygosity ranged from 0.655 in the BAR breed to 0.751 in the COM breed. The low value of genetic differentiation among breeds ($F_{ST}=0.048$) may indicate that these breeds are not differentiated enough and that they may have common history and breeding practices. Recent migration rates (up to the second generation of migrants) and inbreeding coefficients were estimated using the software BAYESASS+. Four out of the five breeds have not received a significant proportion of migrants (BAR, COM, SAR, and VdB). Only for the PIN breed a recent introgression rate from the VdB breed (7.2%) was found. The posterior distribution of inbreeding coefficient ranged from 0.0321 (COM) to 0.0782 (PIN). The Bayesian assignment test, with the software STRUCTURE, showed that BAR and SAR breeds had a more definite genetic structure (proportion of assignment of 92% and 86.6%, respectively), whereas the lowest assignment value was found in the PIN breed (67.1%). Assigning individuals into populations based on the genetic diversity pattern potentially offers unbiased tools in agriculture for the traceability of animals or animal products, for breed confirmation, or for hybridization detection. Because breed assignment for traceability purposes requires a high certainty, extreme care is needed, in particular for undifferentiated breeds ($F_{ST}<0.1$).

C-095**Genetic diversity and phylogenetic relationships among four breeds reared in Sicily using β -lactoglobulin promoter region polymorphisms**

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The aim of this work was to analyze the genetic diversity and the phylogenetic relationships among the Valle del Belice (VDB) breed and the other sheep breeds considered as ancestors, using β -lactoglobulin (*BLG*) promoter region polymorphisms. The *BLG* promoter region of 50 randomly chosen animals from the four breeds (VDB n=20; Comisana (COM) n=10; Pinzirita (PIN) n=10; Sarda (SAR) n=10) were sequenced and aligned, and results showed the presence of 36 single nucleotide polymorphisms (SNPs) and one deletion. The number of polymorphic sites identified showed high variability within breeds.

Nucleotide (π) and haplotype (Hd) diversity were estimated with DnaSP software, whereas PHASE software was used to infer and analyze haplotypes. A total of 22 haplotypes were found in "best" reconstruction and used for phylogenetic analysis with MEGA software (Neighbor-Joining algorithm). The SAR breed presented the lowest genetic diversity ($\pi=0.00355\pm 0.00151$ and $Hd=0.279\pm 0.123$), whereas the COM breed presented the highest one ($\pi=0.00703\pm 0.00055$ and $Hd=0.837\pm 0.076$). Comparing the nucleotide diversity among breeds, the highest value was obtained between VDB and PIN breeds ($\pi=0.00566$), whereas the lowest one between VDB and SAR breeds ($\pi=0.00421$). The phylogenetic tree showed the closest relationship between VDB and SAR breeds. Considering that polymorphisms in the *BLG* promoter region could have a functional role associated with milk composition, the lowest nucleotide diversity between VDB and SAR breeds may be related to a higher similarity of milk composition of these two breeds compared to the others. Further analyses will be conducted on a wider sample in order to confirm the obtained results.

C-096

A Fortran program to optimize selection schemes in dairy sheep

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The efficiency of dairy sheep selection schemes is affected by the rate of artificial insemination (AI) and the application of controlled natural mating (CNM). A Fortran program was developed to compare different dairy sheep selection schemes by stochastic simulations. The aim of this work was to test the program by simulating different AI rates in a situation where controlled NM is applied in each flock with internal rams only. The scheme involved 200 flocks with 200 lactating ewes/flock. Fixed environmental, random permanent environmental and genetic effects (TBV) and random residuals were independently simulated to generate milk lactation yields. TBV generation follows an infinitesimal model ($h^2=0.30$). Genetic evaluation is based on a BLUP-animal model with repeated measures. Planned matings between proven rams and best ewes were simulated within flock by CNM and across flocks via AI. Young rams to progeny test were mated at random. Replacement rate was 25%. Ratio of replacement ewes from proven respect to in test rams was 70:30. Five scenarios (10 replicates each) were investigated with 0%, 25%, 50%, 75% and 100% of flocks involved in AI. In each flock 25% of replacement ewes derived from AI sires. Matings between relatives (with a common ancestor up to grandparents) were avoided. Twenty generations were simulated and selection of males started at generation 4. Annual genetic gain increased as the percentage of flocks performing AI increased, ranging from 13%

to 19% of the genetic standard deviation when no or all flocks were involved in the AI program respectively. Respect to the scenario with no AI, the participation of the 50% of flocks in the AI program allowed increasing the annual genetic progress of 23%. This study confirms that AI is a fundamental tool in dairy sheep selection schemes even when CNM is realised. Further scenarios will be simulated to identify the most appropriate rate of AI considering the costs of the AI program and the impact of using NM rams circulating among flocks.

Acknowledgement

Work funded by the program APQ "Attivazione del Centro Biodiversità al servizio dell'allevamento" of RAS.

C-097

Genetic parameters for antibody response to *Mycobacterium avium* spp. paratuberculosis in natural infected flock

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Mycobacterium avium subsp. *paratuberculosis* (MAP) causes Johne's disease (JD), a granulomatous enteritis of ruminants that has a huge impact on profitability of sheep breeding. MAP has been associated with Chron's disease, a chronic enteritis of man. According to the increasing consumers' demand of healthy products, selection for resistance to animal diseases is a promising approach. To investigate the existence of genetic variation in susceptibility to MAP, blood samples were collected once a year (July) from 1914 animals over a 10-year follow-up period in one MAP-affected sheep flock especially designed to QTL detection. Blood samples were tested by using a commercially available ELISA test for presence of antibodies against MAP. Sheep were considered to be JD positive when with more than one seropositive result or with a seropositive result and dead for JD within the first 4 years of age. Variance components estimation was performed by using the REML method. In order to avoid statistical confounding between the dam genetic and MAP status effects, sire model was used instead of animal model. Given the binary nature, data were analysed with a threshold model including as fixed effect the year of birth to take into account different infection pressures and individual conditions throughout the follow-up period. The pedigree file included 181 individuals, there are 40 sires, 18 sires of sires and 28 maternal grandsires. Heritability of MAP status was 0.28 ± 0.10 . This estimation of heritability is consistent with the existence of genetic variation in

susceptibility to MAP, as documented in cattle.

Acknowledgement

This work was realised in the framework of the European Project 3SR of the FP7.

C-098

Genetic parameters of performance tested traits in Maremmana and Podolica breeds

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Nowadays, performance testing is still the main selection tool for beef and dual purpose breeds, and also rustic breeds as Maremmana (M) and Podolica (P) are selected through this method. The test stations are managed by ANABIC (Italian beef cattle breeds association), responsible for young bulls recruitment (at 9 months of age), test data collection (over 6 months) and selection of young candidate bulls (at 15 months of age). This study was aimed at estimating genetic parameters for tested traits using performance data of almost 15 years of activity. Data on 757 performance tested bulls (300 for M; 457 for P) born from 1996 to 2009 were used in the study. Animals belonged to 13 and 14 contemporary groups (GR) for M and P, while the number of herds of origin (HR) were 23 and 43, respectively. Traits analysed were average daily gain during test (TADG), obtained by linear regression of weight on age, the fleshiness score (FLESH) and the final morphological score (MORPH), both evaluated at the end of test. Preliminary ANOVA indicated significant effects of GR and HR on all traits in both breeds, and a significant effect of the age at end of test as covariate for FLESH and MORPH. Bayesian bi-traits variance component analysis via gibbs sampling using all available pedigree (1959 for M and 2251 for P, respectively) produced h^2 estimates greater for M (0.511 for MORPH; 0.568 for FLESH, and 0.624 for TADG) than for P (0.383 for MORPH; 0.315 for FLESH, and 0.277 for TADG). Positive genetic correlations among traits were estimated for M (from 0.678 between TADG and MORPH to 0.914 between FLESH and MORPH), but milder correlations were obtained for P (from -0.288 between TADG and MORPH to 0.527 between FLESH and MORPH), probably due to greater phenotypic variability and to the lower influence of FLESH on MORPH in P breed as respect to M.

C-099

Efficiency of the partial least-squares genotype imputation method for dense SNP marker maps in cattle

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In genomic selection, direct genomic values (DGV) are evaluated by using genotype information provided by high-density marker platforms. At present costs, the genotyping process is very expensive and problems arise when genomic data extracted from different SNP platforms have to be joined. To overcome these difficulties some imputation algorithms have been recently proposed. These techniques refer to the process of predicting (or imputing) marker genotypes that are not directly collected in some animals. The aim of the present work was to test, with real data, the partial least squares regression (PLSR) imputation method previously developed by using only simulated data. Marker information were provided by the Italian Selmol project. 749 Bruna Italiana sires were genotyped by using the Illumina BovineSNP50 BeadChip. Animals were divided in two groups: 600 old bulls constituted the training population (TP) with all SNP markers considered known. The remaining 149 young bulls were the prediction population (PP) with only 10% evenly spaced SNP considered known. PLSR method was applied to predict the remaining 90% SNP genotypes by using information both from TP and PP. Efficiency of PLSR method was tested by calculating the imputation error rate and the imputation accuracy. The first refers to the proportion of incorrectly imputed genotypes, whereas the second to the correlation coefficient between actual and PLSR predicted SNP genotypes. Imputation effects on DGV were tested by comparing results from the original and the data set with a mixture of actual and PLSR predicted SNP. The mean accuracy and the mean imputation error rate were 0.74 and 0.17, respectively. Moreover, DGV accuracies for milk, fat percentage and protein percentage were 97.5%, 99.3 and 95.2% of the true genomic values, respectively. These results show that the PLSR imputation method properly works also with real data.

C-100

Effect of hormones and growth factors on cell proliferation and expression of urokinase related genes in a bovine mammary epithelial cell line

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The plasmin-plasminogen system orchestrates a variety of extracellular proteolytic events. In the bovine mammary gland it is well known that this system mediates extensive tissue remodeling during involution. However, the role of the plasmin-plasminogen system in proliferation of bovine mammary epithelial cells has not been elucidated in detail yet. The aim of the present study was to evaluate the impact of various hormones and growth factors known to affect mammary physiology and functionality on proliferation and expression of urokinase related genes in bovine mammary epithelial cells. The BME-UV1 cell line has been used as the *in vitro* model of the bovine mammary epithelium. Cells were exposed to insulin, EGF, IGF-1, prolactin and dexamethasone or their combination for 24 h and MTT assay was performed in order to evaluate their effects on cell proliferation. Moreover, plasminogen activator activity was measured by a colorimetric test. Gene expression of urokinase related genes was studied by quantitative PCR analysis. All experiments included three replicates per treatment and were repeated at least twice. The effect of the various treatments was assessed by ANOVA. Fischer's LSD test was used *post hoc*, with a 95% confidence interval. Our results indicate that hormones and growth factors can differentially affect cell proliferation, plasminogen activator activity and expression of the components of the plasmin-plasminogen system. These findings provide more insights into the functional role of plasmin-plasminogen system in the bovine mammary gland.

C-101

Evidence of *HBAY* and *HBBA^{Zebu}* globin genes in Grey Alpine cattle

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Grey Alpine (GA) is a rustic cattle breed traditionally widespread in the North-Eastern provinces of Italy. In the last few years GA cattle are also ranched in areas far away from the center of origin, as highlighted by the presence of herds in Southern Italy. In the present work we investigated both the haematological pattern and the haemoglobin polymorphism in a sample of 58 registered GA cattle from a newly-established herd located in the province of Bari (Apulia, Italy) and composed by animals purchased over time directly from different North-Eastern breeders. By using highly resolving analytical procedures (PAGIF&RP-HPLC), the positively charged Y globin variant, encoded by the *HBAY* gene, was observed. The *HBAY* gene, firstly found in Italian Podolic (IP) cattle, exhibited a 0.15 frequency value as opposed to that of the alternative *HBAIN* gene. We recorded the presence of the common variants A and B of the beta globin system, as well as of the silent mutant *A^{Zebu}*, the frequency of the corresponding *HBBA*, *HBBB* and *HBBA^{Zebu}* genes being 0.74, 0.16 and 0.10, respectively. These results closely parallel results previously observed in IP cattle where both *HBAY* and *HBBA^{Zebu}* were recorded as not rare, none of them being detected on a preliminary screening of Italian Friesian and Italian Brown samples. These findings highlight the need to revise the traditional classification of GA as belonging to the *upland brachyceros* group, also taking into account new developments and phylogenetic evidence from molecular studies. Actually, the presence of identical marker alleles in IP and GA suggest they share a common origin, possibly tracing back to earliest ancestors such as the *Bos primigenius primigenius*. Also it is worth noting that the *HBAY* is associated with decreased haematocrit values, confirming previous findings both in IP cattle and other ruminant species, where positively charged variants have been always related to reduced red cell volume.

C-102**Animal production and emerging issues in veterinary public health and food safety**

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World human population is rapidly growing and the resulting request of food and raw materials is quickly increasing, especially in emerging countries. The animal production industry is a major factor of economic success in many countries; consequently, every problem that can affect this sector may have serious economic consequences. The safety of food of animal origin has become an essential part of the public health debate. With the increase in the international trade, the principles of equivalence, harmonization and transparency of food safety systems become more significant. Risk assessment methods evaluating food safety, based on these principles, become more important as well. In the recent past, the animal production industry has seen, at international level, the devastating effects – among the others – of bovine spongiform encephalopathy in the United Kingdom, foot-and-mouth disease in South America, highly pathogenic avian influenza in Southeast Asia, dioxin in Europe. The effects of globalization and by-product recycling on the supply of raw material for the feed industry, the increasing environmental pollution, the environmental and climate changes, the occurrence of non-epidemic emergencies (earthquakes, floods), the improper or illegal use of drugs and chemicals, are emerging issues having potentially crushing consequences on the livestock industry. Infectious diseases are traditionally one of the main factors able to affect livestock production and trade of animals and their products. The emergence and evolutionary success of infectious agents are related to the selective pressure exerted by environmental factors. The complexity of the modern livestock industry creates opportunities for new infectious agents to emerge exploiting new ecological niches. Similar effects are observed as a result of climate change which are moving the geographical range of vectors and pathogens, once confined to the African continent, towards Mediterranean and Europe. The trade of raw materials and by-products on a worldwide basis leads to the globalization of the risk deriving from the difficulties in tracing and ensuring the routes supplying feed industry. In Europe, the legislation in the field of animal health and food security is defined by common standards aimed at fighting infectious diseases, protecting consumers and animal health and ensuring free movement of goods among member countries. A system of health guarantees based on: 1) internationally-harmonised, transparent and risk assessment-derived legislation; 2) well-organised surveillance and information systems; 3) efficient veterinary services; 4) ready-for-action schemes of preparedness, early warning and response; 5) integration and par-

ticipation of the livestock sector; 6) mechanisms avoiding unfair penalties to breeders; 7) integrated scientific research programs; 8) competent communication systems, is able to ensure that emergencies are adequately controlled and their economic impact mitigated.

C-103**Whole farm systems modelling of greenhouse gas emissions from livestock production systems**

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Whole farm systems modelling can be used to predict the effects of changes in management on greenhouse gas (GHG) emissions and to identify cost effective GHG mitigation options, while ensuring that possible interactions between different GHG are considered. Furthermore, production systems can be evaluated and compared to determine best management practices. Whole farm systems GHG emissions models can be categorised as systems analysis models or life cycle assessment models. However, where the assumptions underpinning the analysis are the same, results should also be the same. In a review of published models of GHG emissions from livestock systems, improvements in liveweight gain for beef production systems was found to be an important mitigation strategy (e.g. Foley *et al.*, 2011). Variations in animal performance levels on GHG emissions from dairy production systems is somewhat less clear, although improved milk yield per cow can reduce GHG emissions (e.g. Beukes *et al.*, 2010). However, if a breeding strategy aimed at improving milk yield per cow resulted in impaired fertility and, consequently longer calving intervals and higher culling rates, overall GHG emissions may increase (Beukes *et al.*, 2010). For both beef and dairy production systems results indicate that increased output per ha through increased intensification can reduce GHG emissions per kg product, provided that excessively high levels of N fertilizer use can be avoided and that overall GHG emissions associated with intensification are offset by higher levels of productivity. In most of the studies reviewed, it was assumed that the soil C balance was in equilibrium. However, Rotz *et al.* (2010) found a GHG offset of 10% to 22% per kg milk where a confinement based dairy system was converted to a pasture based system. Despite the increased flexibility and sensitivity of whole farm systems models to capture farm level activity, concerns and variation relating to data sources, boundaries (i.e., which emission sources are included), emission factors and allocation approaches exist, and they limit direct comparison among studies. Thus, it is critical that assumptions made in this regard are clearly outlined. Nevertheless, in terms of developing and assessing mitigation policies to reduce GHG emissions from livestock

systems, whole farm approaches provide the most robust and comprehensive approach to developing and implementing effective strategies.

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C-104

Survey on the contribution of animal origin foods in Italian dietary habits

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Foods of animal origin (AF) are considered cause of health problems in developed countries while their consumption is growing in developing countries, also to reduce malnutrition. The emphasis to the Mediterranean Diet (MD) and its positive effects suggest a rise of plant derived foods (PF) and reduction of AF. Aim of our study was to estimate the average dietary intakes in 4 Italian areas and to compare them with the frequency of human diseases. Data were obtained by ISMEA/ACNielsen and concern the single foods bought weekly by a panel of 6000 families randomly spread in the whole country. According to daily intake by average person and the typical composition of each food, the energy and nutrient consumption at domestic level were calculated and estimated for a total daily intake of 2241 kcal/d. The average diets of 4 areas were compared with MD and with the frequency of cancer, vascular diseases, diabetes and obesity. Data suggest the difficulties to know the real intake of foods - lack of reliable statistics, data regarding raw materials (e.g. carcass weight for meat, uncured vegetables) - but allow an acceptable estimation of main nutrients contributing to energy in the diet: 15% proteins, 39% lipids and 45% carbohydrates. AF are responsible of 61% of protein, 38% of lipid and 16% of carbohydrate intake. Among areas a lower consumption of AF is observed in the South, while cereals and oils are higher. According to this, the adherence to the MD is slightly higher in South (64%) vs North (57%) and Centrum (61%), but

lower respect to the ideal MD (85%). However, South has less cancer and vascular diseases, but higher diabetes, overweight and obesity. Despite the difficulty to compare dietary and health data, we suggest that present Italian diet is slightly poor in proteins, too rich in fat (62% from PF) and sugars (84% from PF and sweet beverages). This advise a slight reduction of cereals, sweet beverages and non olive oils as well as use of lean meat and low fat dairies.

C-105

Environmental impact of milk production in intensive farming systems: life cycle assessment approach

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Life cycle assessment (LCA) has been shown to be a valuable method for the environmental evaluation of farming systems and has been applied to several agricultural products, particularly in Europe. In LCA the potential environmental impacts of a product are assessed by quantifying the resources consumed and the emissions, at all stages of its life cycle, from the extraction of resources, through the production and transport of materials, to the production process at the farm. The aim of the study was to evaluate the environmental impacts of milk production in intensive farming systems using LCA approach on a sample of 44 dairy farms in northern Italy. Impact categories were: Land use, Non-renewable energy use, Climate change, Acidification and Eutrophication. Functional units were: 1 kg of Fat and Protein Corrected Milk (FPCM); 1 ha of farm land. Data were collected by personal interview with the farmers. Farms involved in the study reared on average 145 (± 91) cows, with a daily milk production of 28.0 (± 3.32) kg FPCM/cow. Average farm land was 49.9 (± 37) ha and stocking density 5.3 (± 2.5) LU/ha. On average, 59% of the total dry matter of cow rations consisted of feed ingredients produced on the farm. The average results for the 5 LCA categories per kg of FPCM were: 2.84 (± 1.02) m² for Land use, 4.98 (± 1.34) MJ for Energy use, 0.93 (± 0.29) kg CO₂-eq for Climate change, 18.2 (± 4.2) g SO₂-eq for Acidification and 12.0 (± 3.1) g PO₄-eq for Eutrophication. Impact indicators from this study are similar to those reported in recent European LC analyses on intensive dairy farms, although the comparison among LCA studies from different countries can be misleading because of local peculiarities and different methodologies. Within the study, LCA indicators allowed to compare environmental impacts of milk production among farms characterized by different levels of intensification (stocking density, milk production, feed self-supply).

C-106**Intestinal digestible protein balance and milk urea in lactating goats**

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Aim of this study was to evaluate, in lactating goats, the effects of diets with two different balances of intestinal digestible protein (PDI), on milk production and milk urea level (MUL). Twenty four lactating Saanen goats, milked once a day, were divided into four pens and fed in a change-over design two diets with different concentration of PDI with Nitrogen (PDIN) or Energy (PDIE) as limiting factors: dietary treatments L (low) and H (high) had, respectively, 102 and 125 g PDIN/kg DM and 102 and 114 g PDIE/kg DM. The diets had the same forage:concentrate ratio (45:55) but different amounts (on as fed) of maize grain and soya-bean meal (maize: 27.3 *vs* 17.9%; SBM: 1.6 *vs* 11.0%, for L and H, respectively). Chemical composition (g/kg DM) of L and H diets were: CP, 149 *vs* 181; NFC, 360 *vs* 323; andNDFom, 398 *vs* 400. Milk yield (MY) and MUL were individually measured, whilst DM intake (DMI) was determined for each pen. Individual DMI was calculated on the basis of MY and body weight according to INRA (2007) and adjusted for intake of the related pen. Data were statistically analysed by SAS-GLM procedure and linear regression analysis was performed between individual MUL and PDIN balance (PDIN intake – PDI requirement). Dry matter intake (overall mean: 2716 g/d), MY (overall mean: 2539 g/d) and milk composition were not influenced by dietary treatments. On the contrary, MUL (mg/dL) resulted higher ($P<0.001$) for diet H (58.3) as compared to diet L (37.1). Dietary N efficiency (milk N/intake N) was greater for the diet with lower CP concentration (0.21 *vs* 0.18) ($P<0.001$). The regression equation between MUL and PDIN balance resulted as follows: $MUL (mg/dL) = 0.151 \times PDIN \text{ bal. (g/d)} + 31.2$; ($n=46$; $r^2=0.20$; $P=0.002$). This equation shows that when the PDIN supply is equal to requirements, MUL is close to 30.

C-107**Effect of the low protein diets on growth performance, backfat thickness and N excretion of heavy pigs**

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Conventional diets used in heavy pig production contain about 14.5% of CP and more than 7 g of lysine/kg feed, which are likely to be in excess considering a protein retention of about 100 g/d. The hypothesis tested in this trial is that a replacement of protein with starch in the diet should not influence the growth performance, improve the back-fat thickness and reduce the N excretion of pigs reared for the dry cured ham production of protected origin (DOP). Four iso-energetic diets (ME = 12.9 MJ/kg) were formulated to contain 14.2, 13.2, 11.9, 11.4% of CP and 45.8 g of lysine/kg CP, achieved by a progressive replacement of soya-bean meal with wheat grain. In two repeated cycles, a total of 160 pigs were housed in 8 pens in groups of 10 individuals. The pigs were divided in 4 groups, balanced for age and BW, and fed one of the 4 diets following a restricted feeding regime. Automated feeding stations measured daily the individual feed intake. Pigs were weighed at the start of the trial (99.3±10.1 kg), every 2 weeks, and before slaughter (168.9±9.0 kg). The two cycles lasted 102 and 112 d. Back-fat thickness was measured by an ultrasound device. Nitrogen excretion was computed as consumption minus retention, assuming a N content of 0.024 kg/kg weight gain. Data were analysed by ANOVA. No significant effects due to the treatment were observed on average daily gain (ADG= 0.650 kg/d; $P=0.66$) and feed conversion ratio (FCR=4.17; $P=0.16$), but back-fat thickness variation during the trial was increased from +7.2 to +8.4 mm with decreasing CP levels ($P=0.007$). The estimated N excretion strongly decreased from 41.6 to 38.4 g/d ($P<0.001$). It was concluded that diets with only 11.4% of CP and only 5.2 g lysine/kg of feed can be applied with success on this production system.

C-108

Wild red deer (*Cervus elaphus* L.) grazing may seriously reduce forage production in mountain grasslands

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While it is well known that overabundance of Red deer may damage forest ecosystems due to intensive browsing, information on the effects of grazing on grasslands is limited. This study aimed at estimating the impact of red deer grazing on the grasslands located in Pian Cansiglio, north-eastern Italian Pre-Alps. Grasslands (383 ha; average elevation of 1000 m asl) are managed for hay/silage production (1-2 cuts per season) and are inside a protected area of 6570 ha that hosts a high density of red deer (estimated at around 30 heads/100 ha with rut census). During April-September 2008 and 2010, DM production and loss due to deer grazing were estimated with exclusion cages (1x1 m;

51 in 2008 and 54 in 2010). Night counts with spotlights were replicated 3 times in 2008 to index local deer density. Data are reported by calculating the means and standard deviation (mean \pm SD), averaged across years. Dry matter production was fairly good for the area (48.9 \pm 14.4 q/ha in the first cut and 26.9 \pm 7.4 q/ha in the second). Losses were significant and did not vary between cuts (8.8 \pm 9.1 q/ha in the first and 8.1 \pm 7.6 q/ha in the second); the variability was extremely high among patches from a minimum of -13 q/ha (DM higher outside the cage) and a maximum of 35 q/ha. ANCOVA and correlation analyses showed that DM losses were negatively related with distance of plots from woodland and positively related with local deer density, that in some plots was as high as 7-8 heads/ha. Deer grazing reduced also CP content of forage (15.6 \pm 4.4% DM inside exclusion cages and 13.8 \pm 3.5% outside), with losses being greater where forage production and CP content were higher.

In conclusion, this study demonstrates that high densities of grazing Red deer may seriously impact on forage production and quality. Further research should elucidate how this impact is influenced by deer behaviour and plant selection, as well by fencing and other possible countermeasures.

C-109**Analysis of different milk payment systems in Veneto region dairy industries and effect on economic value of milk quality traits**

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Aims of this study were to analyze milk payment systems (PS) applied in Veneto region and to estimate the effect on economic value (EV) of milk traits. The PS are usually function of carrier, fat, protein content and somatic cell count (SCC) and there are also new proposals to include novel traits as milk coagulation properties (MCP). On the other hand EV of a trait could be defined as the improvement in profitability resulting from one unit of genetic improvement in that trait, keeping all other traits in the aggregate genotype constant. Seven PS were analyzed, one of which included also milk coagulation time (RCT, min) and curd firmness (a_{30}). For calculation of economic value (EV), average data of Italian Holstein Friesian (HF) dairy cattle reared in Veneto region were set in an equation profit model. The EV of trait x was obtained as the first partial derivative of a profit equation. The EV were more influenced from PS mainly for carrier and SCS ranged from -0.09 to 0.14 €/kg and from -469 to -111 €/point respectively. In PS that include MCP traits, EV for RCT and a_{30} were -6.34 €/min and 3.17 €/mm, respectively. Relative EV (expressed as% in a milk quality sub-index), MCP traits account for 6.2%. A more harmonized milk PS should be defined as well selection index that account for requirements of dairy industry.

Acknowledgement

Funds by Distretto Lattiero Caseario del Veneto: LATSIER project, DGR 1966 del 15/07/08, Mis. 2A - Regione Veneto.

C-110**Influence of the storage of milk on cheese yield**

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The effect of the time interval between milking and cheese-making on cheese yield (CY) was investigated using a laboratory micro-manufacturing method. Raw bulk milk from the morning milking of cows reared at the experimental farm of the University of Padova was collected on the first day of the trial and stored in a refrigerator at 4°C. Five cheese-making sessions (CMS) were carried out during 5 consecutive days by the same operator. For each session, 2 water baths (WB), each supplied with 8 stainless containers (SC), were used; this resulted in 80 cheeses produced dur-

ing the whole trial (8 replicates x 2 WB x 5 CMS). The CMS consisted in the coagulation of 500 ml of milk per SC previously inoculated with starter, the cutting of the curd, the separation of the whey through drainage, and the pressure and weighing of the curd to compute CY. An analysis of variance was performed on CY according to a linear model that included the fixed effects of CMS, WB, SC, and all first order interactions between the main factors. The effect of CMS was significant ($P < 0.001$) in explaining the variability of CY, suggesting that the storage of milk before cheese-making had an impact on CY. Further studies will deal with the estimation of repeatability of CY over CMS.

C-111**Pecorino cheese quality as affected by milk storage time**

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A significant increase in unit costs in cheese making is recorded when ewe's milk production is lowered related to seasonal lambing. According to law, milk can't be stored for more than 48 hours, and this forces dairies to withdraw and to process even small quantities of milk. The aim of this study is to verify the possibility to extend the milking - cheese making gap up to 72 hours, without affecting the quality of the cheese. The study was carried out in a herd of about 320 Massese sheep, milk was stored in refrigerator at +4 °C in separate milk churns, seamless, to ensure constant temperature and pecorino cheese was made in the farm. Milk samples (for chemical, technological and microbiological analysis) were collected after 24 hours (A), 48 hours (B) and 72 hours (C) of storage times in relation to daily production and to storage capacity. After cheese making of A, B, C milk, 3 cheese moulds were collected from each thesis, on which chemical and microbiological analysis were carried out after 2, 15, 30 days of ripening. Results were processed with SAS statistical package using t-Student test. Milk C showed no increase in Total bacterial count, Total Coliforms, *Escherichia coli*, and *Staphylococcus coagulase positive* and the same was true for the 30 days cheese compared to those produced by A and B milk. Milk C showed an increase in titratable acidity and free acidity and a significant reduction in PT due to continued proteolytic action, while high contents of TL and SS and SCC depend on the stage of lactation. Chemical composition of the cheese didn't seem to be linked to milk ones. Organoleptic quality of cheese was not linked to storage time of milk, because the technological characteristics of milk A, B, C, show no significant differences (r : 12.25; 12.32; 11.35 min and for a_{30} : 36.55; 38.29; 36.33 mm respectively). Therefore, it is possible to extend storage time of milk up to 72 hours, as long as with steady temperature, without adverse effects on pecorino cheese quality.

C-112

Antioxidant properties of donkey milk

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In the present work the well-known beneficial donkey's milk (DM) characteristics to human health have been investigated, focusing the attention on anti-oxidant properties. For this purpose, vitamin C, lactoferrin, lactoperoxidase activity and total antioxidant power (PAO) were determined on milk whereas the reactive oxygen metabolites (ROM) and PAO concentrations were evaluated on blood. The trial was carried out on 10 pluriparous jennets. Sterile blood and milk samples were collected during three different periods of lactation. Microbiological tests, SCC (by a fluoro-optoelectronic method), PAO and ROM determinations were performed by commercial kits. Quantitative determination of lactoferrin from DM was determined by an HPLC method, lactoperoxidase activity was evaluated by a continuous spectrophotometric method, vitamin C content determination was performed using a commercial kit. The obtained data were subjected to the analysis of variance (ANOVA, SPSS). Mean values of the investigated parameters \pm standard errors were calculated. No significant differences were observed for ROM, SCC and neither blood or milk PAO (respectively, 4.45 ± 0.1 mmol/L; $50.9 \pm 19.9 \times 10^3$ /mL; 166.2 ± 4.1 and 55.5 ± 3.2 μ mol HClO/mL). The fresh DM contains 57 mg/L of vitamin C, similar to that of human milk but higher compared with that of cow's milk. The lactoferrin concentration in DM is 0.080 ± 0.0035 g/L, very close to that obtained for mare, bovine but lower if compared with human milk. Mature DM contains low but detectable LPO activity (0.11 ± 0.027 mg/L) similarly to value obtained from human milk. Concluding, for the first time were determined the PAO and ROM reference range in DM, furthermore the high content of vitamin C in DM confirms the important nutritional characteristics of this product.

C-113

Receiver-operating characteristic curves for SCS in Valle del Belice dairy sheep

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Although direct bacteriological assay is the recommended method for mastitis diagnosis, other indirect methods are widely used, one of which is milk SCC. SCC, and therefore SCS, has indeed been widely recognized as a selection criterion for improved mastitis resistance. The definition of a SCC threshold able to detect healthy from diseased animals might be used to define selection strategies for mastitis resistance. SCC diagnostic capability may be assessed without having to commit to a single threshold with Receiver-Operating Characteristic (ROC) curves. Data consisted of 1357 records for SCS and infection status on 684 Valle del Belice sheep from four flocks. The continuous data were categorized using ROCKIT 1.1.β2 software to estimate the ROC curve using an ML curve for categorical data. The ROC curve was used to select thresholds (for whole SCS, and SCS for both minor and major pathogens), yielding the optimal mix of False Positive and False Negative Fraction, given a specific diagnostic test, infection prevalence (P), and costs assigned to false positive, false negative, true positive, and true negative results. Highest percentages of correctly classified results are determined by the point on ROC curve (i.e. the best operating point) where a straight line with a slope equal to the 'cost' function f is tangential to the ROC curve. In our case, this occurs in a point with $Se=0.52$ and $1-Sp=0.13$ for whole SCS ($SCS=2.84$), $Se=0.45$ and $1-Sp=0.13$ for SCS_MIN ($SCS=2.88$), and $Se=0.61$ and $1-Sp=0.03$ for SCS_MAJ ($SCS=3.33$), suggesting that the ROC measures are preferable to using a single critical value which may lead to confusion when different criteria are used.

C-114

Lactation curves of Bianca Val Padana and Italian Friesian dairy cows reared in the same herds

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Aim of the study was to evaluate milk production and lactation curve parameters of Bianca Val Padana (BVP) and Italian Friesian (IF) cattle when reared together in the same herds. To this aim, 8485 test day records from 774 lactations of 103 BVP and 367 IF cows from 7 farms in the province of Modena were used; 2 herds were free stalls and TMR feeding (FS-TMR group) and 5 were tie stalls and traditional feeding (TS-TF group). Data of milk, fat, protein and lactose production (kg), fat, protein and lactose content (%) and somatic cell count (SCC) were submitted to analysis of variance, using a linear model with breed, housing type, test day, herd, season of parity, number of lactation, class of days in milk (DIM) and interactions as fixed factors. Nonlinear regression analysis according to Wood was calcu-

lated starting from the LS means of the interaction of class of DIM, breed and housing type. The interaction between breed and type of housing has always been significant ($P < 0.05$), except for milk fat percentage and SCC. In particular daily milk, fat, protein and lactose productions were lower in TS-TF compared to FS-TMR, but the reduction was significantly higher ($P < 0.05$) in IF than in BVP (kg 4.91 vs 3.63; kg 0.219 vs 0.137; kg 0.164 vs 0.117; kg 0.258 vs 0.179, for milk, fat, protein and lactose, resp.). The percentage of protein showed an opposite trend in the two breeds depending on the type of housing (BVP +0.12%, IF -0.02% from FS-TMR to TS-TF; $P < 0.05$). The lactation curves were best fitted by Wood model in IF than in BVP for both housing types (higher R^2 , lower RSE). Week at peak and persistency of lactation were higher in BVP in TS-TF group than in FS-TMR, as in IF the contrary was observed. In conclusion BVP seem to be better adapted to TS-TF rearing conditions than IF.

C-115

k-casein B content of herd milk for Parmigiano-Reggiano cheese

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Various studies have confirmed that the B variant of k-casein positively affects cheese yield, milk technological properties, easy processing of milk and cheese quality. The aim of this research is to monitor a significant number of farms to highlight the contents of k-casein B in bulk milk of individual farms in the Parmigiano-Reggiano production area and provide guidance for an update of the parameters to be considered in the milk quality payment system in use. Samples of bulk milk from the morning milking were collected from 502 farms, chosen as to represent the actual distribution of farms in the Parmigiano-Reggiano production area, distributed into the 5 provinces and plain, hill and mountain. On each sample the analyses of the milk quality payment system and the determination of k-casein B by "Testkappa" were performed. For each farm the type of feeding, cattle number, breeds and milk production were considered. The average percentage of k-casein B on total casein was 2.39 ± 2.02 ; it showed a wide variability, ranging from 0.10 to 12.56. The analysis shows that this parameter is influenced by genetic factors such as cattle breed and not by environmental factors. The k-casein B mean value for the Italian Friesian breed was low (1.75%, k-casein B on total casein), nevertheless 23 Friesian

farms showed values equal or higher than farms using breeds, like the Italian Brown, that traditionally are provided with more k-casein B. The k-casein B content affects both curd firming time and curd firmness. It should be noted, in this way, that the "k-casein B content" parameter can provide additional information on the quality of milk for Parmigiano-Reggiano cheesemaking.

C-116

Mineral content and salt equilibria of milk from wild ungulates species

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Mineral content and salts equilibria of individual milk samples collected from 19 hinds (*Cervus elaphus*), 27 fallow does (*Dama dama*) and 5 roe does (*Capreolus capreolus*) were studied. Milk samples of both hinds and fallow does were collected from animals reared in outdoor enclosure, whereas those from roe does were collected from wild subjects. Prior milking, 10 IU of oxytocin were injected by intramuscular administration to facilitate milk ejection. After 1-2 minutes, females were handily milked. Total phosphorus (P) and soluble P were determined by means of colorimetric method on milk and skimmed milk ultrafiltrate (cut off 30,000 D), respectively. Total contents of calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K) were determined on full cream milk by atomic absorption spectroscopy (AAS). Soluble fractions of Ca and Mg were determined on skimmed milk ultrafiltrate (cut off 30,000 D) by AAS. Total nitrogen and non-casein nitrogen were determined by the Kjeldahl method on milk and on pH 4.6 acid whey of milk, respectively. Total contents (mg/100g) of Ca, P, Mg, Na and K of milk resulted (mean \pm SD) (*C. elaphus*, *D. dama*, *C. capreolus*) 273 ± 35 ; 245 ± 48 ; 413 ± 99 , 189 ± 15 ; 163 ± 19 ; 312 ± 13 ; 18 ± 2 ; 29 ± 6 ; 23 ± 2 ; 41 ± 7 ; 44 ± 9 ; 45 ± 3 and 126 ± 6 ; 126 ± 22 ; 140 ± 16 , respectively. Soluble fractions (%) of Ca, P and Mg were (*C. elaphus*, *D. dama*, *C. capreolus*) 27 ± 6 ; 27 ± 3 ; 22 ± 3 , 30 ± 6 ; 28 ± 5 ; 30 ± 1 and 56 ± 10 ; 58 ± 9 ; 49 ± 15 respectively. The contents of Ca and P associated to the casein micelle (g/100 g of casein) were (*C. elaphus*, *D. dama*, *C. capreolus*) 3.12 ± 0.50 ; 2.69 ± 0.59 ; 3.85 ± 0.60 and 1.99 ± 0.35 ; 1.65 ± 0.28 ; 2.56 ± 0.24 , respectively. The mineralisation degree of the casein micelle of roe does milk was higher compared with the milk of both hinds and, in particular, fallow does.

C-117

Volatile organic compounds and free D-amino acids in ewes' milk as affected by temperature treatments and total microbial count

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The aim of the study was to approach the analytical determination of chemical markers able to define the quality of sheep milk. Gas chromatographic determination of free amino acid enantiomers and headspace solid-phase microextraction, gas chromatography mass spectrometric detection of volatile compounds were applied to ewes' milk samples characterized by different

total microbial counts (TMC) and temperature treatments. Twenty bulk raw milk samples were obtained from Sardinian ewes and treated, before the final freeze-storage at -80°C , as follows: 1) raw milk; 2) raw milk refrigerated at 4°C for 48 h; 3) raw milk treated at 37°C for 3 h; 4) raw milk treated at 37°C for 3 h and refrigerated at 4°C for 48 h. Correlations between TMC and D-amino acids or volatile compounds were evaluated by Pearson's analysis. Student-Newman-Keuls was used as *post hoc* test for comparison of the means among different groups, P value of ≤ 0.05 was considered as significant. Positive correlations between TMC and D-Ala ($P < 0.05$) and between D-Ala and D-Asp ($P < 0.05$) were found. D-amino acids contents in milk groups did not produce any significant difference. D/D+L amounts of D-Ala, D-Pro and D-Asp were lowest in group 1. D-Ala was highest in group 3 and 4, whereas D-Asp was highest in group 2. Changes in D-Met were opposite to D-Ala and D-Asp variations, with the highest values observed in group 1 and the lowest ones in group 4. Concerning the volatile fraction, ketones resulted the most abundant class of compounds, followed by aldehydes and alcohols. Sulfur and acid compounds were less represented. No correlations were observed between TMC and volatile compounds in milk; similarly, comparison of means of volatile compounds within groups was not significant.

C-118**Behaviour, glycaemia and growth performance of underweight piglets**

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The increase of litter size caused a parallel increase in the incidence of underweight piglets. Aim of the study was to investigate behaviour, glycaemia and growth performance of underweight piglets during pre-weaning. The study lasted 7 wks and was carried out in a commercial pig farm considering 50 underweight piglets (body weight: 742.8±36.9 g/head) and 53 regular weight piglets (body weight: 1941.8±42.9 g/head). Piglets belonged to 5 litters per treatment. In order to record individual data all newborns were earmarked. At day 0, 7 and 21 from birth piglets were weighted and at day 0 and 7 glycaemia and individual reactivity with a back-test were measured. Data were processed using a linear model which considered the effect of body weight, gender (male *vs* female) and mortality (died *vs* survived). Gender did not affect the parameters considered in the study. Glycaemia at birth was lower in underweight piglets than in regular weight animals ($P<0.001$) but at 7 days of age glycaemia between regular weight and survived underweight piglets was similar. The mortality rate of underweight piglets was very high when compared to those of regular weight (30% *vs* 0%, $P<0.001$). Underweight piglets that died from 0 to 7 days of life compared to those survived underweight ones had a lower body weight at the birth (644 *vs* 789 g, $P<0.001$) and glycaemia (40 *vs* 59 mg/dL). The growth rate of underweight piglets was always lower ($P<0.05$) and differences in body weight between the two treatments were continuous ($P<0.001$) so that piglets lighter at birth were still lighter at weaning. Behavioural observations carried out during the back-test (n. of squeals, grunts and escape attempts) did not show differences between underweight and regular weight piglets. Results suggest that glycaemia could be a good indicator for the potential survival of underweight piglets and it is likely a parameter to monitor for the development of feeding strategies that can reduce mortality.

C-119**Farmers' opinion about the welfare of beef cattle and how it should be assessed on-farm**

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This study aimed at investigating the opinion of Italian beef cat-

tle producers on animal welfare and the ways it should be assessed. A survey was carried out by a questionnaire distributed to a sample of 69 intensive fattening units (1/farm) located in the Veneto region (North-eastern Italy). The sample covered a wide range of farms in terms of size (from 45 to 5000 bulls fattened/year) and of type of pen floor adopted for cattle housing. After gathering descriptive information on farm characteristics, questions were addressed to investigate farmers' opinion on animal welfare. The questions were organized in a way such that the interviewee had to rank given options according to the importance he attributed to each of them. Data were submitted to descriptive statistical analysis and one-way nonparametric tests were performed for location differences across farm size, farmer age and type of floor. No significant distribution differences were detected. Results showed that healthier and less restrained animals were considered as the main meanings attributed to the term "animal welfare" by the farmers. Two opened questions tried to understand in which way farmers recognize a good or a poor cattle welfare status. Most answers were clustered to animal-based measures such as behaviour, health and feeding rather than to performance or to farm facilities. The little importance given to the growth performance as a welfare indicator might be linked to the lack of a frequent gain control for beef cattle, different from what occurs with the milk yield in dairy farms. It must be noticed that these two open questions were left blank respectively in 29 and 37% of the questionnaires. This underlines that a large part of the farmers is still lacking of a proper training to identify relevant welfare indicators on-farm. Data on cattle health, housing structures, feeding plan and number of medical treatments were ranked in descending order as main measures to be included into an ideal welfare assessment scheme for beef cattle farms. Farmers are aware of the current EU research to set up of a standardized welfare assessment scheme which is perceived as a helpful tool to improve farm management rather than a duty or a rule to comply with.

C-120**Opinion of the Italian pig farmers about animal welfare**

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The study aimed to investigate the attitude of the Italian pig farmers towards the topic of animal welfare considering the effectiveness of legislation in force and the alarm toward its possible update. In the survey a group of 30 farmers belonging to the Italian breeders association has been interviewed by a questionnaire consisting of 20 closed-ended questions. Five questions regarded factual information such as type of production, size of

the farm, number of person working with the animals. The remaining questions regarded specifically the farmers' opinion on animal welfare. The farms were categorized according to the type of production: 8 farms addressed to reproduction, 10 to fattening, and 12 had both types of production. Statistical analyses were performed using χ^2 tests with Marascuilo procedure to verify the association between responses (practices and beliefs) and type of production. When the association was significant ($P < 0.05$) odds ratios were calculated. Results showed that farmer as manager of the farm is the main responsible of the animal welfare (83% of farms) whereas employees attend more frequently courses on animal welfare (53 vs 33% of farmers) likely because they work in contact with the animals. Sources for farmers' update on the animal welfare topic are: farmers' associations (34%), farm vet (26%) and technical journals (17%). Despite the relevance of rooting for the welfare of pigs, none of the farms adopted straw as enrichment whereas they used no-rooting materials such as metal chains. According to the farmers' opinion, the production of new regulations on pigs welfare have low probability to occur due to the detailed legislation already in force for all pig categories. However they don't consider the strong impact on the legislator from the negative public opinion on mutilation and castration. In case of a change of the legislation farmers considered alternative types of farrowing crates and floor very difficult to manage.

C-121

Probiotic supplementation of milk replacer: effect on lamb performance

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In many specialized dairy flocks, lambs are early separated from their dams and fed commercial milk replacer to increase volume of milk destined to dairy farm for cheese-making. This study was undertaken to assess the effect of milk replacer containing *Lactobacillus acidophilus* and a mix of *Bifidobacterium longum* and *Bifidobacterium lactis* on growth performance, immune response of lambs. Forty male Comisana lambs were divided into four groups of ten each and fed different milk source: maternal milk (MM), milk replacer (MR), milk replacer with *Lactobacillus acidophilus* supplementation (MRL), and milk replacer with a mix (1:1) of *Bifidobacterium lactis* and *Bifidobacterium longum* supplementation (MRB). The experiment lasted 40 days (lambs were 2 to 42 days of age). Lambs were weighed with an electron-

ic scale at weekly intervals, *in vivo* cell-mediated immunity and humoral immune response were determined during the trial. Lambs of MRB group showed the highest lymphocyte mitogen response to phytohemagglutinin (PHA); lambs of MR group displayed the lowest antibody response to ovalbumin, whereas no differences emerged among dam-suckled lambs and lambs receiving milk replacer supplemented with probiotics throughout the trial. Feeding regime affected interleukin (IL) expression: the IL-1 β /IL-10 and IL-6/IL-10 ratios showed the highest levels in MM lambs evidencing that in lambs fed maternal milk the regulatory process by IL-10 was less active than in the other groups. The addition of *L. acidophilus* and a mix of *B. longum* and *B. lactis* to milk replacer sustained the growth performance of lambs suggesting an important role of probiotics in modulating the immune function via both immune stimulation and immune regulation.

C-122

Production and behavioural traits of heavy pigs subjected to two illumination periods

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According to latest EFSA's recommendations (2007) on the welfare of fattening pigs, the aim of the present trial was to give a contribution to the still debated problem concerning pig requirements in terms of environmental illumination. Forty hybrid pigs (26 kg BW) were homogeneously allocated into two experimental groups (20 animals per group) each containing four replications of five pigs: group LL (Long Lighting) in which pigs were exposed to a 16-hour/day light period and group SL (Short Lighting) in which, in compliance with minimum legislative standard, pigs were subjected to a 8-hour/day light period. For both groups the light intensity was 40 lux, equivalent to the minimum mandatory level. Pigs were fed on the basis of their metabolic BW up to a maximum of 3.3 kg/pig/day. From 100 to 160 kg BW pigs were monthly videotaped over a 24-hour period in order to assess, by scan sampling, their behaviour. Growth parameters and post-mortem outcomes were submitted to analysis of variance (GLM procedure) with lighting duration as the main effect. For non-parametric data (behavioural traits), Mann-Whitney test (NPAR1WAY procedure) was used. During the first phase of the experiment (0-118 days of trial), animals receiving the longer illumination period (LL group) showed a significant ($P < 0.01$) improvement of average daily weight gain and a reduction ($P < 0.05$) of feed conversion rate, leading to a higher ($P < 0.05$) final body weight. At slaughtering, despite similar lean meat yields (assessed by F-o-M), pigs on LL group produced heavier

carcasses and heavier hams ($P < 0.01$). With respect to behavioural traits, pigs receiving the longer illumination period were more calm as demonstrated by a higher percent of lying behaviour and by a lower incidence of pseudo-rooting behaviour ($P < 0.01$). From a general standpoint it can be concluded that a prolonged photoperiod with a low light intensity could have some positive effects both on production and behavioural traits of pigs.

C-123

Some aspects of behaviour of the progeny of a bull clone over the pre-weaning period

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The current welfare assessment in cloned domestic animals and their progeny is mainly based on data related to the health status. Behavioural is an useful indicator of ability of domestic animals to cope with environmental challenges. Behavioural studies are thus integral to animal well being assessment. In the present work, the behaviour of progeny (n. 7) of a Holstein Friesian bull clone (CLp) housed in individual cages was monitored and compared to that of calves (n. 9) obtained from the corresponding nuclear transfer-donor bull (DONp). Animals were monitored at 4 age periods (within the 2nd, 3rd, 4th and from 5th to 8th weeks of age). Behavioural observations were conducted within two daily time bands (morning time band, starting the 2nd h from morning milk meal, MB; afternoon time band, around milk meal, AB), each lasting three hours, by scan sampling every 3 minutes. Data were analyzed by mixed models for repeated measures; bull, time band, age and time of the day and their interaction were considered as fixed factors and calf as random factor. Overall, no anomalous behaviours, such as oral stereotypies, were observed. No difference was found between CLp and DONp for the other considered behaviours. Calves rested more in MB than AB; licking structures, eating solid food, chewing, and standing inactive in the cage and at the feeder, resulted more expressed in AB than in MB, reaching maximal values around milk meal time. Non-nutritive sucking was only observed after milk meal consumption. Standing inactive at the feeder, chewing, sniffing, and number of vocalizations decreased with age, while eating solid food increased. In conclusion, progeny of bull clone showed a behavioural repertoire comparable to that normally observed in dairy calves.

C-124

Body growth and haematological profile of heifer calves sired by a bull or its clone

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The aim of this paper was to compare welfare-related aspects of the female progeny of a bull sire with that of its clone. Sixteen Friesian female calves, 9 daughter from a tested bull (BULL) and 7 from its somatic cell nuclear transfer clone (CLON) were monitored from birth to 36 wk of life. Body weight (BW), wither height (WH), and heart girth (HG) were measured: at birth, 4, 8, 12, 16, 20, and 24 wk of age. Blood samples were taken from jugular vein at 24-48 h, 1, 2, 3, 4, 8, 12, 16, 20, 24, and 36 weeks of age, to be analysed for haematological profile and blood glucose concentration. At the same time, rectal temperature (RT) was recorded. Data were evaluated using a mixed model, taking into account the repeated measures in time on the calf. For each variable, different covariance structures were tested, choosing the best according to the Akaike's Information Criteria. Significant was set at $P < 0.05$, and a trend was considered for $P < 0.10$. At the end of 24 wk, CLON daughters were smaller in WH than BULL daughters. Around 20 wk of age, a trend in BW differences (with CLON daughters lighter than BULL daughters) was also confirmed from difference in HG. There were no differences in RT due to sire effect. Blood glucose decreased in both groups during the first 4 wk of life; at birth, only a trend for higher blood glucose in CLON daughters was recorded. Total leukocytes count did not differ between progenies. Circulating lymphocytes had a trend to be lower in CLON than BULL daughters. The neutrophil:lymphocyte ratio had a trend to be higher in CLON than BULL calves. No difference was demonstrated for erythrocyte features, whereas mean platelet volume had a trend to be lower in CLON than BULL progeny. From these results, there is no difference between progenies from BULL and CLON that may suggest welfare problems in the first 6 mo of life.

C-125**Heat stress modulates glucose partitioning in mice**

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Alteration of glucose metabolism in heat stressed animals is reported, but mechanisms involved are not clearly understood yet. In order to improve knowledge on the effect of heat stress on glucose partitioning an *in vivo* study on mice was performed. Seventy-two male C57BL/6 mice, 7 weeks old were used. After 14 d adaptation period under thermoneutral conditions (TN: 22°C), the mice were subdivided into 3 groups of 24 each. One group was kept under TN, a 2nd group was exposed to heat stress (HS: 35°C) and a 3rd group was kept under TN and fed restricted (FR). FR was calculated on the basis of the food ingested under HS. The experimental period lasted 5 d. Daily food intake and rectal temperature (RT) were measured. At the beginning and after 2 and 5 d of the experimental period (ExP), blood samples were taken and 6 animals of each group were sacrificed to obtain adipose tissue (AT), liver (L) and muscle (M) samples. Plasma insulin, glucose and NEFA were determined. Tissues were analyzed to determine mRNA of insulin receptor (IR), Hsp70.1, GLUT4 and GLUT2 by real time RT-PCR. β -actin was used as housekeeping gene. At d 0 and 5 of ExP a glucose tolerance test (GTT) was performed on 6 mice (15 h starved) of each group. HSed mice had higher RT compared to TN and FR mice, and lower food intake compared to TN. Hsp70.1 gene expression was higher under HS than FR and TN. Blood glucose was lower under HS and FR compared to TN. NEFA were lower in HS compared to TN and FR. Insulin was higher under HS compared to TN and FR. HSed mice had greater glucose disposal compared to their TN and FR counterparts. IR was upregulated in HSed and FR mice in AT and M compared to TN, while IR mRNA showed the highest levels in L of HSed mice. GLUT4 and GLUT2 mRNA levels were higher in HSed mice compared to FR and TN mice. This *in vivo* study provides a first evidence that HS is directly responsible for the increase in insulin sensitivity and for a modulation of glucose partitioning in peripheral tissues.

C-126**Haematological profile of periparturient Friesian and Modenese cows**

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The aim of this research was to assess haematological variables in two bovine breeds, Italian Friesian (F) and Modenese (MO) during the peripartum period, when defence mechanisms are generally depressed. Thirty pluriparous cows, 17 F and 13 MO, raised together in a free stall barn under identical environmental conditions, were sampled for jugular vein blood from 5 wk pre- to 4 wk post-calving. Blood was collected before feed distribution in the morning, and analysed for haematological features with an automatic analyser. Data were evaluated using a mixed model, taking into account the repeated measures in time on each cow. For each variable, five covariance structures were tested to choose (according to the Akaike's Information Criteria) the one which best fitted the data. Breed, time (T-5, T-4, T-3, T-2, T-1, T0, T1, T2, T3, and T4 wk from calving), and breed \times time interaction effects were tested. Differences were considered significant at $P < 0.05$; a trend was considered when $P < 0.10$. The breed affected the percentage of monocytes (M) and mean corpuscular haemoglobin concentration (MCHC), whereas a trend was evidenced for circulating neutrophils count (N), mean corpuscular volume (MCV), and platelets (PLT). Time affected all the measured parameters. Breed \times time interactions were evidenced for white blood cell count (WBC), N, and mean platelet volume (MPV), whereas a trend was observed for neutrophil:lymphocyte ratio (N:L), mean corpuscular haemoglobin, MCHC, and red cell distribution wideness. In both breeds, WBC increased during T-3 to T-1 wk from calving, mainly due to a rise in N. Means of WBC and N were lower at T0 and T1 in MO than F cows; at T0, N as % of WBC, and N:L were lower, whereas the percentages of L and M on WBC were higher in MO than F cows. Results indicate that MO cows seem less susceptible to peripartum physiological stress as described by the change in circulating leukocyte populations and, as consequence, by the N:L ratio.

C-127

Comparison of European sea bass (*Dicentrarchus labrax*) from organic and semi-intensive rearing systems

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The study aimed to compare biometric and rheological traits and chemical composition of sea bass coming from the organic and semi-intensive rearing systems of an Italian fish farm (Veneto Agricoltura). The two systems differed for the diet, organic and conventional, while water conditions and stocking density were similar. After 18 months of rearing, 40 specimens (20 per rearing system) were slaughtered by immersion in ice slurry and analysed the day after catch. Biometric traits, dressing percentage and fillet pH, CIE L*a*b* colour and texture were measured. Chemical composition and fatty acid profile of the diets and sea bass fillets were analysed. Individual data were submitted to ANOVA by GLM procedure of SAS. Sea bass showed similar weight at slaughter (447 vs 421 g; P>0.05) and no difference in biometric traits or dressing percentage. Fillets showed similar texture profile, pH and L* and a* indexes, while b* index was higher in organic sea bass than in semi-intensive sea bass (1.39 vs 0.54; P<0.01). Fillet proximate composition did not change, while fatty acid profile differed according to the composition of the diets: in particular, organic sea bass showed higher proportion of saturated fatty acids (FA) (23.7 vs 22.2%), monounsaturated FA (33.4 vs 31.3%) and n-3 polyunsaturated FA (22.7 vs 14.4%) and a lower proportion of n-6 polyunsaturated FA (16.0 vs 29.1%) compared to semi-intensive sea bass (P<0.001). The n3/n6 ratio was higher in the organic fish (1.42 vs 0.49; P<0.001). In conclusions, differences between organic and semi-intensive sea bass concerned their nutritional value and exclusively depended on the feeding regime.

C-128

Growth performance of different rainbow trout (*Oncorhynchus mykiss*) strains reared in Trentino (Northern Italy)

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We will present some results of an ongoing project aimed at analysing the major biotic and abiotic factors that influence the rainbow trout (*O. mykiss*) productive yield in the Trentino region (Northern Italy). Five trout strains were compared for their overall farming performance and suitability to be reared in the local farms. Eyed-egg samples of different strains were obtained from local and foreign suppliers. In the first part of the trial, the strains were compared in terms of egg size, hatching and growth rates up to the parr stage in a single farm and the intraspecific genetic variability was assessed using a microsatellites technique by analysing DNA extracted from random samples of caudal fin tissue. Significant among-strains differences in growth performance were found after 8 rearing months. In the second phase of the trial, parrs of each strain were divided into 4 lots. Then the lots of the 5 strains were transferred to 4 selected Trentino trout farms to carry out a performance test up to a market size of around 0.7 kg. Regardless of the location, all fish lots were kept at the same density and were fed the same commercial trout feed, six days a week. Individual weight and length were measured on random samples of 100 fish per lot every 2 months. At the same time, major water parameters were registered in the different farms. Specific growth rate (SGR), thermal growth rate (TGR), condition factor and feed conversion ratio (FCR) were calculated. Statistical analyses were performed with SAS and STATISTICA 9.0. The growth graphs fit quadratic equations and average daily gain ranged from 1.46 to 1.77 g. Among-strains differences in growth performance, condition factor, feed conversion and age at sexual maturity were found at market size.

C-129

Comparative growth of the Mediterranean mussel (*Mytilus galloprovincialis* Lamarck, 1819) reared in three coastal areas of Sardinia

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Mussel culture is the most important aquacultural activity in Sardinia (Italy). Small specimens (42.5±3.1 mm shell length, 2.3±0.6 g wet meat weight) of *Mytilus galloprovincialis* of the same origin (Taranto) were grown in suspended culture from April to October 2010 in three different Sardinian coastal lagoons: 1) Calich, 2) Porto Pozzo, and 3) Tortoli. Several morphometric variables (i.e., shell length, shell height, wet shell weight, wet meat weight, and wet total weight) were measured monthly in 60 mussels from each of the experimental groups. During the same period, a number of hydrological variables (i.e., temperature, salinity, pH, and dissolved oxygen) were monitored

fortnightly at each lagoon, whereas chlorophyll a and seston content in the water column was determined monthly. A two-way analysis of variance was used to test for differences in mussel shell length and condition index (CI = wet meat weight/wet total weight \times 100) between 'sites' and 'sampling periods'. Post-hoc multiple comparisons were performed using the Student-Newman-Keuls test. After six months, mussels grown in the Calich lagoon showed a significantly higher mean shell length (66.2 ± 4.7 mm; $F(2, 1062) = 117.3$, $P < 0.001$) than those from Porto Pozzo (63.5 ± 3.2 mm) and Tortoli (61.6 ± 2.7 mm). Similarly, at the end of the trial, mean CI value was significantly higher in *M. galloprovincialis* specimens from the Calich lagoon (60.9 ± 5.3 ; $F(2, 1062) = 847.5$, $P < 0.001$) than in those from Porto Pozzo (51.4 ± 3.9) and Tortoli (49.4 ± 4.4). Significant differences due to 'sampling period' and interaction 'site \times sampling period' were also detected. The influence of the abiotic variables on mussel growth is reported and discussed.

C-130

Study of the intestinal microflora of gilthead sea bream (*Sparus aurata* Linnaeus, 1758) reared in off shore floating cages

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Fish gut microflora is important for aquacultured species because it reflects the bacterial flora of the rearing environment, the type of diet and plays a role in the health and quality of adult fish. Previous quantitative studies on intestinal bacterial flora of gilthead sea breams reared in floating cages showed low bacterial loads. The aim of this research was to investigate the qualitative composition of the intestinal microbiota of gilthead sea breams reared (Autumn 2008) in off-shore floating cages, located in the Alghero bay along the Sardinian coast, by means of phenotypic tests, ARDRA and sequencing of the 16S rRNA gene. This polyphasic study aimed to identify the dominant heterotrophic bacteria of the gut to evaluate the microbiological quality of fish and bacterial biodiversity to detect a link with the rearing system. The qualitative microbiological analyses highlighted the presence of 19 different ARDRA phylogenetic groups and five dominant bacterial species were identified by sequence analysis: *Pseudomonas fluorescens* (22%), rainbow trout intestinal bacteria (14%), *Myroides profundii* (14%), *Psychrobacter* sp. (11%), *Cryseobacterium* sp. (2%) and other Gram-negative (37%). These microbial species are described as typical of both aquatic environments and fish gut. *Pseudomonas fluorescens* is also described as principal aerobic Gram-negative on food products, whereas rainbow trout intestinal bacteria as a component of the gut of rainbow trout. *Psychrobacter* sp. and *Cryseobacterium* sp., have been commonly isolated from skin, gills, and have proved to be dominant in the gut of Atlantic cod fed bioprocessed soya-

bean meal. Our results indicate the presence of non pathogenic bacteria in the gut of these fish, a good microbiological quality, good rearing system hygienic conditions, a high bacterial biodiversity and a close link between gut microflora and the type of sea bream's diet.

C-131

Optimization of reproduction and larval rearing of red porgy, *Pagrus pagrus* (L.)

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The aim of the trial was to set up a reproduction and larval rearing technique for red porgy (*Pagrus pagrus*), considered one of the most important emerging species for marine aquaculture. A total of 100 (40 females, 60 males) four years old red porgy, weighing 700 ± 300 g, were equally subdivided in 2 indoor tanks (V1, V2) (50 m^3 each) connected to a recirculation system. Water was maintained at 37 ppt of salinity and temperature increased from 14 to 26°C according to a 8 months gonad maturation programme. Photoperiod was 15 h light:7 h dark obtained by means of fluorescent lamps (150 lux at water surface). Fish were fed *ad libitum* fresh mussels, defrost squid and fresh anchovy. Spawning started without hormonal injection at the beginning of April and eggs were collected in baskets and incubated in two 1,000 l circular tanks at the density of 1.5 kg/tank (=1,500,000 eggs) at $15 \pm 0.5^\circ\text{C}$, with a water flow of 0.4 l/min and moderated aeration. Larvae hatched after 3 days of incubation and were transferred in 2-10 m^3 tanks at $19 \pm 1^\circ\text{C}$. Larvae were exposed to a daily photoperiod of 24 h light from day 3 to 17, and 16 h light from day 18 to 60. Light intensity measured at the water surface was 120 lux. Dead larvae were daily removed and counted. Feeding protocol included enriched rotifers from day 3 to day 30, while enriched *Artemia nauplii* were added from day 20. From day 30, inert diets of different size (200-300, 300-500, 500-700 μm) were supplied. Trial lasted 60 days, when the fingerlings were completely weaned: at this time, mortality was calculated. Eggs had a fertilization rate of 90% (V1) and 85% (V2), while the hatching rate was 75% in V1 and 73% in V2, respectively. The development of swim bladder started on day 10 and lasted until day 15. Larval mortality was very low ranging from 2% (day 3) to 0.5% (day 7) and decreasing with the time; total mortality was 18-20%.

C-132

Effects of vacuum pack on the shelf life of live mussels (*Mytilus galloprovincialis*)

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Italy is one of the main mussel producer Country in the EU, but this production is not matched by development of processing techniques able to maintain product quality and facilitate storage and utilization along the supply chain. Changes in consumer's habits suggest the need for processed products with longer shelf-life also for traditional seafood like mussels. This research was aimed to compare the effect of under vacuum package (SV) with traditional plastic net (RT) on live mussel shelf-life. The effect of the storage method was evaluated on chemical, physical and microbiological parameters of *M. galloprovincialis* kept at 3°C±0.5 for 16 days. Statistical analysis was used to compare the collected data. In a first trial, groups of 15 mussels were checked in duplicate in terms of mortality, intervalvar liquid release, edible portion water holding capacity and microbiological counts to evaluate the effect of the innovative and traditional package at day 1, 3, 6, 9, 13 and 16. Vacuum storage resulted able to extend live mussel shelf-life: mortality started earlier in RT group (day 6 of storage) relatively to the SV group (day 9 of storage). In a second trial, the effect of byssus removal was tested. Four groups of 15 mussels were daily sampled for 13 days. Mussels kept under vacuum with byssus (SVBS) exhibited lower mortality compared to RT groups or under vacuum after byssus removal (SVDB). Microbiological counts and pH values of the edible parts, although modified during storage, did not reveal significant differences between groups SVBS and SVDB. The research shows the efficacy of vacuum packaging to extend live mussel shelf-life in comparison with the traditional plastic net while preventing product contamination and body fluids loss.

Acknowledgement

Research funded by Regione Friuli Venezia Giulia (LR26/05).

C-133

Effect of dietary grape seed extract in common sole

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Sole farming is hampered by poor growth and high sensitivity to stress and infectious diseases. Hence, supplementing diets with health promoting additives such as antioxidants could be worth studying to improve the growth performance of this species. Grape seed proanthocyanidin extract (GSPE), a natural polyphenolic antioxidant, has been used as a human food supplement for health promotion, but few data are available on its application in animal nutrition and none in fish. The present study was aimed at investigating the effect of supplementing a commercial dry extruded feed formulated for sole (Diet E) with GSPE at a dose of 50 mg/kg (Diet E+GSPE) on the growth performance, blood plasma lipid profile and total antioxidant activity of *Solea solea*. Two hundred eighty four fishes (mean weight 38.1±0.6 g) were randomly distributed in 8 rectangular troughs (0.37 m²) at 3.7 kg/m² stocking density, and fed at 1% body weight/day over 147 days, according to a single-factor experimental design with 4 replicates per diet. Survival rate, feed conversion ratio (FCR) and specific growth rate (SGR) were calculated per group at the end of the trial, whereas plasma levels of total, HDL, LDL-cholesterol, triglycerides and total antioxidant activity were measured at days 87 and 147 on 8 fish samples per treatment. All dietary groups had similar, high survival rates (95.8 vs 97.1%, P>0.05). Diet E+GSPE resulted in a slight but significantly impaired SGR over diet E (0.28 vs 0.31, P<0.05), but in the same FCR (P>0.05). Blood lipid profile was also unaffected by the dietary treatment, while the addition of GSPE to the diet resulted in significantly increased antioxidant activity in plasma (P<0.05) suggesting the usefulness of grape seed extract in promoting resistance to oxidative stress in common sole.

C-134

Growth performance and fatty acid metabolism in European grayling (*Thymallus thymallus*, L.) fed diets differing in n-3 and n-6 PUFA levels

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A feeding trial was carried out to evaluate the growth performance and fatty acid metabolism of juvenile grayling (*T. thymallus*, L., Adriatic strain) in response to three diets supplying varying levels and ratios of essential fatty acids (n-3 PUFAs, 7, 12, 8 g/kg; n-6 PUFAs, 5, 10, 12 g/kg; n-3/n-6 ratios, 1.6, 1.2, 0.6). Each diet was fed to visual satiety over 9 weeks to triplicate fish groups, kept at 13.5±0.4°C, each consisting of 25 specimens (average wgt. 12±0.3 g). Regardless of the dietary n-3 PUFA content, fish growth and feed efficiency improved (P<0.05) by increasing dietary n-6 PUFA level or decreasing n-3/n-6 ratio. Based on indi-

vidual fatty acid mass balance, grayling was shown to be able to convert dietary linolenic and linoleic acids to their LC-PUFA homologues (DHA and ARA), due to substantially high activities of $\Delta 6$, $\Delta 5$ desaturases and elongases which were significantly increased in fish fed diets supplying n-3 and n-6 PUFA mostly in the C18 form. It was concluded that in the grayling, as in certain salmonid fish species studied to date, dietary requirements for essential fatty acids could be met either by their C18 precursors or LC-PUFA forms and that quantitative requirement for both n-3 and n-6 PUFA are of the same order of magnitude.

Acknowledgement

Research funded by Ente Tutela Pesca Regione Friuli Venezia-Giulia.

C-135

Sea bass intestinal oligopeptide transporter (PepT)-1: molecular cloning and mRNA quantification during fasting and refeeding

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Oligopeptide transporter 1 (PepT1) is an integral plasma membrane protein responsible for the uptake of dietary di- and tripeptides in cells. It transports peptides against a concentration gradient by coupling the movement of substrate across the membrane, with the movement of protons down an inwardly directed electrochemical proton gradient. A unique feature of PepT1 is the capability of sequence-independent transport of nearly all possible di- and tripeptides, including differently charged species. Accumulating evidence indicates that PepT1 may have a major role in fish nutrition. However, to our knowledge, there is no information regarding the response of such transporter at the mRNA level to different feeding regimens in fish. In the present study, as part of our ongoing work on elucidating genes involved in compensatory growth induced by refeeding, we have now isolated a full-length cDNA representing the PepT1 in the European sea bass (*Dicentrarchus labrax*). A total of 3014 bases including a 5'-untranslated region (101 bp), an open reading frame (ORF) (2184 bp), and a 3'-untranslated region (729 bp) were detected. We also analyzed fasting- and refeeding -induced changes in the expression of PepT1 mRNA, using a one-tube two-temperature real-time RT-PCR and the mRNA standard curve method. Our results revealed that PepT1 was highly expressed in the proximal intestine with much lower levels of expression in the gill, brain, heart, liver and spleen. Nutritional status significantly influenced PepT1 mRNA copy number in the proximal intestine, inducing a down-regulation during prolonged fasting (35 days) and an up-regulation during the recovery from fasting. These

findings offer new information about the dietary regulation of PepT1 mRNA level in sea bass, and support a role of this membrane transporter protein in promoting sea bass compensatory growth induced by refeeding.

C-136

Evaluation of very early weaning of common sole (*Solea solea* L.) using commercial diets: implications on performances, larval quality and tank hygiene

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A study on common sole (*Solea solea* L.) larvae was carried out in order to assess the potential for a very early weaning onto commercial microdiets before the end of metamorphosis. Four days post hatching (dph) larvae were randomly allocated in twelve flat bottom (0.64 m²) 280 L square tanks (2000 larvae tank⁻¹) connected to a recirculating system. Four experimental weaning feeding regimes (FR) were used in triplicate: Control (FR-C) where larvae are fed *Artemia nauplii* and *metanauplii* with dry feed until 27th dph. The others FR provided live feed until 13th dph, employing the same diet used in FR-C (AgloNorse Ewos, Norway – FR-A), Gemma Skretting, France (FR-G) and O.range Large INVE, Italy (FR-O). The trial lasted until 33rd dph. Data were subjected to statistical analyses using a General Linear Model, the ordinal regression and one-way analysis of variance (ANOVA) with Tukey's post test. No significant differences among groups were observed in survival at the end of the trial. Groups subjected to very early weaning (FR-O, A and G) showed lower weight and length when compared to control, but that did not result in a delay in the onset of metamorphosis. Fatty acids profile of larvae was influenced by the FR. The total number of *Vibrio* spp. was higher in FR-C tanks at 21 dph, probably due to the presence of live feed in rearing water. Larval quality measured at the end of the trial by the salinity stress test was not influenced by treatments. In conclusion, very early weaning in sole affects growth potential but not survival and susceptibility to stress.

C-137

Effects of cooking on lipid content and fatty acid profile of meagre (*Argyrosomus regius*) fillets during the first three days of storage

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Despite its high qualitative intrinsic traits, farmed meagre (*Argyrosomus regius*) is still a niche species, very little known to European consumers. Fish fatty acids (FA) profile is characterized by moderately high C20:5 (EPA) and C22:6 (DHA) ω -3 polyunsaturated fatty acid (PUFA) contents, having beneficial effects on human health. Storage and cooking process can cause lipid loss, enough to modify raw fish nutritional content. Aim of this study was to describe the effect of cooking on lipid content and on FA profile of meagre during the first 3 days of storage (1°C). Fish (n. 72) were sampled from cage and tank in the farm "Il Vigneto" (Grosseto, Italy), analyzing 6 fish from cage and tank per day. Left and right fillets were analyzed raw and steam cooked (10 min), respectively. Total lipids content and FA profile were determined. Data were analysed by PROC GLM procedure including fish weight, rearing method, sampling date, storage day and the interaction (rearing method x storage day) in the model. Fillets showed a low lipid content (2.59%). During storage cooking yields decreased (96.5 to 94.9%; $p < 0.05$) whereas lipids and FA true retention (%TR) did not show a significant decrease. PUFA %TR ranged from 98.9 to 91.0%, without showing a significant decrease for both ω -6 and ω -3 FA groups, however. Among ω -3 fatty acids, DHA showed the highest average %TR (97.58% at the 3rd day). Steam cooking did not seem to affect the lipid content and FA profile of meagre in the first 3 days of storage. Since any losses of lipid and fatty acid contents can be important for a lean fish, as the meagre is, further researches should be directed to individuate conditions of cooking with lower impacts in the nutritional quality of flesh.

C-138

Dicentracin gene expression in European sea bass (*Dicentrarchus labrax*) fed with a Bio-Mos[®] supplemented diet

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Concern over the use of dietary antibiotics in aquaculture has encouraged the industry to search for alternatives that both enhance performance and afford protection from disease. Bio-Mos[®], derived from the outer cell wall of a specific strain of yeast *Saccharomyces cerevisiae* (Alltech Inc, USA) is a product that fits these criteria. Here, we present data on the impact of a Bio-Mos[®] supplemented diet on the mRNA copy number of the antimicrobial peptide dicentracin, whose transcript regulation has not yet been explored in fish. We analyzed Bio-Mos[®]-induced changes in the expression of sea bass (*Dicentrarchus labrax*) dicentracin, using real-time RT-PCR technology with which the gene expression can be absolutely quantified using the standard curve method. All data were statistically compared using analysis of variance ($P < 0.05$). Our results revealed that 30 days of feeding fish with diets containing Bio-Mos[®] supplemented at either 3‰ or 5‰ significantly increased the dicentracin mRNA copy number in the head kidney. Furthermore, the mRNA copy number in fish fed at 3‰ was significantly higher than that of the group fed at 5‰ for the same period of feeding Bio-Mos[®]. A longer feeding period (60 days) did not further increase the dicentracin transcript levels as compared to the values recorded after 30 days. However, the transcript levels in fish fed at 3‰ proved to be significantly higher than those of the control after 60 days of feeding. These findings offer new information about the response of antimicrobial peptides at the transcriptional level to diets supplemented with immune response modulators, and support a role of Bio-Mos[®] in promoting sea bass non specific immune system.

C-139**Digestibility of whole ear corn silage used as ingredient of fattening diets for pigs**

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Fibrous feeds allow to increase satiety, health status and welfare of fattening pigs. Aim of the trial was to determine the digestibility of whole ear corn silage (ES) included at two levels (15 and 30% DM, ES15 and ES30 respectively) in substitution of corn meal (C) in diets for pigs at different growth stages during the heavy fattening cycle. Six pigs were used in three digestibility trials, which were performed at 35, 85 and 135 kg average live weight of animals. Each trial was divided into two 14 d experimental periods, with the total faeces collected in the last 5 d. In each trial, during the first experimental period the pigs were grouped in three pairs, which received the three diets (C, ES15, ES30). In the second period the pigs of each pair were assigned to the two diets not received in the first period. Data were analysed per trial, according to a two period change-over design $y = \mu + \alpha_i + \beta_j + \delta_k + \varepsilon_{ijk}$, (μ = overall mean; α β δ = period, diet and animal effects, i , j and k , 2, 3 and 6, respectively). The substitution of C with ES increased the NDF content of diets from 12.8 to 16.0 and to 19.2% DM in diets C, ES15 and ES30. The DM content of faeces decreased regularly with the ES inclusion in all trials, but the reduction was statistically significant only in the third trial (29.9, 27.0 and 26.4% DM, respectively for C, ES15 and ES30, $P < 0.05$). In the first trial, the inclusion of ES affected negatively the digestibility of organic matter (88.9, 86.3 and 84.8%, respectively for C, ES15 and ES30, $P < 0.01$) and NDF (50.6, 45.5 and 41.2%, respectively for C, ES15 and ES30, $P < 0.01$). In the two subsequent trials, digestibility was not modified by ES inclusion. In conclusion, ES can be used at levels of 30% DM at intermediate and final fattening cycle without appreciable depressions of digestibility and with a reduction of faecal DM content. Further investigation have to assess the impact of ES on fattening and slaughter performances of heavy pigs.

C-140**Effect of rumen-protected lysine on milk for Parmigiano-Reggiano production**

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The aim of this study was to evaluate the effects of the utilization

of rumen-protected (RP) lysine on milk production and quality as well as cheese yield. Ninety three cows were divided in 2 groups (homogenous for milk yield, age and lactation number; one pen per group) and received a basal diet added (65 g/cow/d of Aminoshure-L; Balchem, USA; Lysine group) or not (Control group) with RP lysine. The basal diet was based on alfalfa hay (7 kg), mixed hay (7 kg), sugar beet pulp (1.5 kg), straw (1 kg), soya-bean meal 44% (1 kg), heat treated soya-bean (1 kg) and mixed concentrates (12.3 kg). Mixed concentrates contained 48.6% corn, 16.3% wheat, 16.3% barley, 12.2% bran, 2% trace elements and vitamins, 1.4% NaHCO₃, 0.7% NaCl, 0.6% yeasts, 0.3% CaCO₃, 0.2% MgO and 1.2% RP methionine; both experimental diets were fed as TMR and provided the cows with the same level of CP (14% DM), starch and fibre. The experimental period was divided in 3 phases (each one of 30 days); dietary treatments were switched 2 times. The lysine/methionine ratio in the Control and Lysine groups was 2.82:1 and 3.06:1, respectively. Individual milk samples were collected after 14, 21 and 28 days of each phase and analyzed for fat, protein, casein, cells and urea; moreover, weekly, milk from the two experimental groups was separately converted into cheese, in order to evaluate cheese yield after 24 h. Results were analyzed by ANOVA following a switchback design in which the pen acts as its own control. Milk yield and quality were not influenced by treatment; throughout the trial, daily milk production was (mean \pm SEM) 32.54 \pm 0.26 kg while milk protein, casein and fat content were 3.50 \pm 0.01%, 2.77 \pm 0.05% and 3.69 \pm 0.02%, respectively. Cheese yield at 24 h did not differ between treatments and was 9.1 kg of cheese/100 kg of milk. Present results show that increasing lysine availability in duodenum of dairy cows by feeding RP lysine had no effect on milk production and quality and cheese yield.

C-141**Modulation of concentrate use in the diet of lactating Sarda ewes: technical and economical aspects**

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This research studied the modulation of concentrate supply to lactating ewes to improve farm economical efficiency. A flock of Sarda ewes in early lactation was divided into two small flocks (groups) of 48 ewes each. All animals grazed on pasture for 8 hours/d and received hay *ad libitum*. One group (T) was supplemented with the traditional dose of 600 g/d of concentrate per ewe, whereas the other group (P) received 200 g/d of concentrate per ewe. During 157 DIM, BW was measured monthly, and milk yield (MY) and composition every two weeks. Ewes' daily intake was estimated as Pulina *et al.* (1996), whereas cumulative flock milk yield (MY) was obtained as sum of sold MY and 4.5 kg of milk

equivalent per kg of BW change. During the trial, feed cost was 120 €/ton of DM for pasture and hay and 266 €/ton of DM for concentrate, whereas milk price was 0.65 €/L. The income over feed cost (IOFC) was used to compare the two technical treatments. Five scenarios were also simulated varying the feed cost, the milk price and the ewes' milk production level. Flock T showed a higher cumulative MY than flock P (8204 vs 7237 L of milk sold; 8564 vs 8113 L of equivalent milk produced, respectively). Milk quality did not differ significantly between flocks. Flock T had also higher supplementation cost, lower BW change, higher IOFC (+159 €) in terms of milk sold, and lower IOFC (-176 €) in terms of equivalent milk than flock P. In the simulated scenarios, flock P was more efficient than flock T only with low milk price (<0.5 €/L) and high concentrate price (+20%), whereas flock T was constantly better than flock P with MY>1.5 L/ewe per d. Pasture for dairy ewes is an important and cheap feed source, but low concentrate doses (200 g/d per ewe) may lead to unfavorable farm performance at high production levels in short-time choices. Potential economical benefits of low concentrate use can be reached with medium to low production levels, even if the positive effect of BW gain on subsequent lactation must be quantified.

C-142

Production response to changes in total mixed rations composition in dairy cattle using a random regression test-day model

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The objective of this study was to estimate the effect of diet on cow production to improve herd productivity from a nutrition point of view. A random regression animal model was fitted to a dataset with 46,531 test-day (TD) milk yield records from 3554 cows in 27 herds in Ragusa province recorded from 2006 through 2008. In these herds total mixed rations (TMR) were collected quarterly from March 2006 through December 2008 and analyzed for ash, crude protein (CP), soluble nitrogen, acid detergent lignin, neutral detergent fibre (NDF), acid detergent fibre (ADF), and starch. These parameters are important for the correct energy balance, the digestive efficiency of cow, but also to optimize diet formulation and feeding strategy. The model was fitted using ASREML and included parity, age at calving, year at calving, and pregnancy stage as fixed effects. Random effects were herd×TD, sire and maternal grandsire additive genetic effect, and permanent environmental effect modeled using 3-order Legendre polynomials. The variables describing TMR chemical composition were included in the model one by one as fixed effects by associating TD records to the closest TMR fed to animals. The effect of TMR chemical components was

modeled as an interaction with days in milk (DIM) using a 9-order Legendre polynomial. Conditional Wald F statistic on fixed effects revealed significant effects ($P<0.001$) for ADF, NDF, CP, and starch, and their interactions with DIM on milk, fat and protein yield. Based on these results, a multivariate analysis was performed where CP, NDF, and starch were simultaneously included in the model. Both analyses revealed that diet composition influenced production responses depending on lactation stage. Results from this study demonstrated that CP, NDF and starch content in TMR affected individual lactation curves for milk, fat and protein depending on stage of lactation. To accomplish feeding the appropriate ration based on DIM and production, farms should group cows accordingly to minimize under and over feeding.

C-143

Effect of linseed and stoned olive pomace supplementation on fatty acid composition and oxidative stability of milk from grazing ewes

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In the present study, the effect of different sources of vegetable fats in grazing dairy ewes on milk fatty acids and its oxidative stability was investigated. Fifteen Comisana ewes (160±14 DIM), grazing a natural pasture, were fed 600 g/head/d of three experimental concentrates: L (25% rolled linseed: 11.8% EE, 15.0% CP and 24.1% NDF on DM basis); OP (27% dried stoned olive pomace and 4% olive oil: 10.9% EE, 15.1 CP and 23.7% NDF on DM basis); LOP (12.5% rolled linseed, 13.5% stoned olive pomace, 2% olive oil: 11.4% EE, 15.4% CP and 24.4% NDF on DM basis). The experimental period lasted four weeks after fifteen days of adaptation. Production was recorded weekly and milk samples collected for chemical composition. Oxidative stability of milk lipid fraction was measured by dosage of 2-thiobarbituric acid-reactive substances (TBARS) expressed as mg of malondialdehyde (MDA) per L of milk at 0 and 72 hours of storage at 4°C. All data were analyzed as repeated measures using a mixed model. Treatment had no effect on milk production and chemical characteristics of milk. CLA content increased by 168% and 158% in L and LOP milk, respectively, whereas C18:3 n-3 increased by 143% and 72% in L and LOP milk, respectively, in comparison to OP milk. Dietary treatment did not affect the level of MDA in milk after 72 hours of conservation (0.832, 0.602, 0.590 mg/L for treatment L, OP and LOP respectively). Adding linseed alone or in combination with olive pomace to grazing ewes diets can improve the nutritional value of milk without compromising its oxidative stability.

C-144

Feeding growing rabbits with low dietary protein level and high soluble fibre to starch ratio

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To evaluate the effect of decreasing dietary protein and replacing starch with soluble fibre on digestive physiology and performance, 246 hybrid rabbits (782±53 g LW) weaned at 33 d of age were divided into six groups and fed ad libitum until slaughter (76 d) six iso-ADF diets. The diets varied according to two protein levels (15.2% and 16.2%) combined with three soluble fibre to starch ratios (0.2, 0.6 and 1.5) obtained by replacing starch (20.9 to 9.1%) with soluble fibre (4.8% to 13.6%). Data were submitted to ANOVA by GLM procedure of SAS. The diet with 16.2% CP and 0.2 soluble fibre to starch ratio determined the highest mortality rate (17.1 vs 1.7%; $P<0.001$) compared to the other diets. When dietary CP increased, the digestibility of dry matter (61.5% to 62.6%), gross energy (62.0% to 63.0%) and aNDF (29.8% to 32.3%) significantly ($0.001<P<0.10$) raised and caecal volatile fatty acids (VFA) increased (59.0 to 68.4 mmol/L; $P=0.01$). Growth performance and slaughter traits were not affected by the protein level. When soluble fibre to starch ratio raised, the digestibility of dry matter and gross energy did not change, while the digestibility of aNDF (26.4 to 35.2%) and ADF (16.7 to 26.7%) linearly increased ($P<0.001$). Caecal VFA concentration (56.0 to 67.3 mmol/L) and acetate proportion linearly increased, while butyrate and valerate proportions decreased ($0.01<P<0.05$). Growth performance was similar among groups, while at slaughter the proportion of the gastro-intestinal tract linearly increased (17.7 to 18.4%; $P<0.01$) without affecting dressing percentage. Meat pH linearly decreased and L*, a* and b* colour indexes increased ($0.01<P<0.05$). In conclusion, feeding rabbits with low-protein and high-soluble fibre to starch ratio diets reduced mortality, improved digestive efficiency and intestinal environment without affecting growth performance.

C-145

Effect of feed supplementation with dried leaves of sage (*Salvia officinalis*) on performances and meat quality traits in rabbits

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Some plant extracts are accounted as phytobiotic and their potential for reducing enteric disorders or improving meat lipid fraction stability is known. Sage could be used as a natural additive for rabbit for its chemical composition. The study investigated the effect of feed supplementation with dried leaves of two sage varieties, differing in their essential oil profile, on performances, carcass and meat traits of rabbits. Forty-five male Bianca Italiana rabbits 30 d old (830±30 g) were allotted to 3 diets: C (control DM 889 g/kg; CP 169 g/kg; CF 171 g/kg), SE (C+1% *S. officinalis* cv. Extrakta) and SL (C+1% *S. officinalis* subsp. *lavan-dulifolia*), and individually fed *ad libitum* for 48 d. Feed intake (FI) and BW were recorded every 10 days, while health status and mortality were monitored daily. At the end, 8 rabbits per diet were randomly chosen and slaughtered without fasting. Carcass traits were determined and *m. longissimus thoracis* (LTM) sampled. Proximate composition, lipid profile and colour of raw LTM, cooking losses and shear force of cooked LTM were evaluated. Data were analyzed by one-way ANOVA and differences by Duncan's test. Diet significantly improved average daily gain (ADG g/d) (25.79 and 28.22 vs C 21.31) and FI (g/d) (96.45 and 95.16 vs 81.61 C) in SE and SL groups, respectively. Final BW was significantly higher in diet SL (2212 g) than SE (2094 g) and C (2000 g). Mortality experienced was not different and equal to 13% in C and 6% in both SE and SL. No difference were observed in meat quality traits even if some fatty acids (C12:0; C17:1 and C20:5n3) showed significant differences between diets. In conclusion, sage supplementation could be an interesting phytobiotic for rabbits, but further studies are needed in order to define optimal dietary inclusion level and elucidated the mechanism of action.

C-146**Effect of dietary mannanoligosaccharides on volatile fatty acids and ammonia production in rabbit caecum**

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To evaluate the effect of mannanoligosaccharides (MOS) on caecal fermentation characteristics, 144 thirty-five days old hybrid Hyla rabbits were equally allotted to 3 groups fed on the same diet without additives (control group), with antibiotics (ANT group: colistin sulphate, 144 mg/kg; tylosin, 100 mg/kg; and oxytetracyclin, 1000 mg/kg) or with MOS (1 g/kg of diet). The caecal content of 10 rabbits per group was collected at 62 days and analyzed for volatile fatty acids (VFA) production (gas-chromatography method) and ammonia content (colorimetric method). Rabbits from the MOS group had a higher ($P<0.01$) level of acetate in the caecal content (39.93 vs 34.21 and 23.09 mmol/l, respectively for MOS, control and ANT groups). The MOS group also showed a higher ($P<0.01$) production of propionate than the ANT group (4.14 vs 2.09 mmol/l, respectively), while no significant difference was found for the control group. No significant difference for butyrate production was detected among the three groups. Caecal microflora of the MOS group rabbits also had a higher fermentative activity in respect of protein source, as demonstrated by the higher ($P<0.01$) productions of branched chain fatty acids (sum of isovaleric and isobutyric acids on total volatile fatty acids). Ammonia content was significantly higher ($P<0.01$) in the control group than in the others (5.31 vs 3.97 and 3.69 mmol/l, $P<0.01$, respectively for control, MOS and antibiotic groups), indicating a better synchronism in protein and carbohydrate source fermentation for MOS and antibiotic groups. MOS are able to improve structural carbohydrate fermentation, in particular of the cellulose, as well as a better synchronism in caecal fermentations and can be used as an alternative to antibiotics during rabbit growth.

C-147**Effect of dietary supplementation of mannanoligosaccharides on meat fatty acid composition of rabbit**

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To evaluate the effect of mannanoligosaccharides (MOS) on meat quality, 512 sixty-day-old hybrid Hyla rabbits were randomly divided among 4 groups fed on the same basal diet, supplemented respectively with apramidine (ANT) at 50 mg/kg and MOS at 0.5, 1.0 or 1.5 g/kg diet. At 82 days, 16 rabbits per group (sex ratio 1:1) were slaughtered. After 24 h of refrigeration at 4°C, the left hind leg was dissected from the carcass and the meat was used for fatty acid analysis. Total lipids were extracted from the homogenized meat samples using the chloroform/methanol method and fatty acids were determined by gas liquid chromatography. Data were analysed by one way ANOVA to test the effect of dietary treatments. Fatty acid composition of rabbit meat showed slightly differences among groups. In particular, MOS_1.0 and 1.5 groups had a significant lower content of palmitic acid than ANT group (27.19 and 27.80 vs 30.78% of total lipids, $P<0.05$). ANT group had the highest ($P<0.05$) content of stearic acid (0.87%) followed by MOS_0.5 (0.78) and, together, MOS_1.0 and 1.5 groups (0.67). MOS_1.0 and 1.5 groups had a higher ($P<0.05$) percentage of oleic acid (31.46 and 30.73%, respectively) than the other two groups. ANT group showed also the highest ($P<0.01$) proportion of saturated fatty acids and both MOS_1.0 and 1.5 groups had a higher proportion of monounsaturated fatty acid than ANT group. However, the percentage of polyunsaturated fatty acids was unaffected by dietary treatments. As in rabbits, the fatty acids synthesized from carbohydrates are mainly palmitic, oleic and stearic acids, while polyunsaturated fatty acids result from the ingested lipids, our hypothesis is that the effect of MOS on intestinal microflora can induce the selection of bacteria with different ability than that selected by antibiotics in saturation/desaturation of organic acids, affecting fatty acid composition of rabbit meat.

C-148**Effect of sex on meat quality of Maremmano wild boar (*Sus scrofa majori*) x Duroc sow reared outdoors**

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Production of hybrids (wild boar x swine) can be a valid way to give back some properties to pig meat, lost in commercial swine breeding. This study was designed to investigate the effects of gender on nutritional and physicochemical meat properties derived from crossing the Maremmano wild boars (*Sus scrofa majori*) with sows of Duroc breed. Sixteen hybrids, 8 barrows (B) and 8 gilts (G) of 6 months of age (live weight: 57.3±0.3 and 56.9±0.2 kg for B and G, respectively; P>0.05), were reared outdoors and were fed ad libitum commercial diets (13.1 MJ of EM/kg and 160g of CP/kg) until slaughter, at 11 months of age (live weight: 128.0±0.1 and 121.0±0.1 kg for B and G, respectively; P<0.01). Hot and cold carcass weights were recorded and dressing percentages were calculated after dressing and chilling at 2-4°C for 24 h. The *Longissimus lumborum* (LL) and *Longissimus thoracis* (LT) muscles were removed from chilled carcasses and the following analyses were carried out: in the LL muscle dry matter, protein, lipid, ash, cholesterol, and selected minerals (Ca, K, Mg and Na); in the LT muscle collagen properties (collagen and crosslink concentrations) and Warner-Bratzler shear force (WBSF). Mean values of data have been compared using the Student's t-test. Compared to G, B had higher (P<0.01) carcass weight, dressing percentages, lipid (2.31 vs 1.93% of wet muscle) and cholesterol (71.47 vs 68.81 mg/100 g of wet muscle) contents. Gender had no significant effect on the dry matter, protein, ash and mineral contents, WBSF, and IMC properties. Results from the present study suggest that cholesterol and fat contents may be sensitive to gender.

Acknowledgement

The research was funded by Ministry of Education, University and Research, Italy. 2007 (Cofin PRIN Prot. 2007WA4872_001)

C-149**Effect of low-protein diets on carcass traits and ham quality of heavy pigs**

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A trial was conducted to evaluate the effect of low-protein diets on carcass traits and ham quality of heavy pigs. A total of 160 animals, divided in two cycles (80 individuals per cycle), were blocked by weight in a randomized block design (10 pigs per pen and 2 pens per dietary treatment). Four iso-energetic diets (ME=12.9 MJ/kg) were formulated to contain 14.2, 13.2, 11.9, and 11.4% of crude protein (CP) and 45.8 g of lysine per kg of CP, achieved by a progressive replacement of soya-bean meal with wheat grain. Animals were slaughtered at 9 months of age (average BW of 168.9±9.0 kg) in the same abattoir and on a single day. During the first hour after slaughter, hot carcass weight was recorded and all carcasses were dissected into typical commercial cuts. Carcass yield (%), backfat (mm), lean (%) and fat cuts (%) were measured. After the 24-h chilling period, fresh hams were trimmed to produce the typical round-shape of San Daniele ham. At the end of the trimming line, hams were weighed and evaluated for overall fatness and marbling. All left hams were then cured according to the San Daniele ham procedure. Measures of ham quality traits were: fat covering measured by ultrasound (mm), iodine number, initial weight (kg), intermediate weight (kg) and final weight (kg) after the ripening process. All traits were analyzed by ANOVA. No significant effects due to the treatment were observed on carcass traits except for the incidence of lean cuts: carcasses from pigs fed with lowest CP had lower incidence of lean cuts than carcasses from pigs fed highest CP ration (61.3% vs 62.3%, respectively; P<0.05). Backfat was higher for pigs fed with low than with high CP rations (32.1 mm vs 27.7 mm, respectively; P<0.05). Results suggest that pigs can be fed with 11.4% of CP without detrimental effects on carcass traits and ham quality.

C-150**Fatty acid composition of subcutaneous adipose tissue of raw and seasoned ham in three PDO dry-cured ham typologies**

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The quality and quantity of subcutaneous adipose tissue (SAT) of pig thighs own a great importance for the needs of processing industry. The aim of this research was to study the fatty acid composition of SAT of fresh thighs, destined for the production of three different typologies of PDO dry-cured hams according to the methods of Parma (PP), San Daniele (PSD) e Toscano (PT), obtained from animals reared in the same farm, and to verify, at

the end of seasoning period (12 months), the effect of the type of production. Forty-eight thighs coming from 24 Italian Landrace x Large White heavy pigs (carcass weight 130.3 ± 11.5 kg) slaughtered in three different days, were sent, left and right in turn, to the three processing plants. Iodine value and fatty acid composition were measured on samples of fresh ($n=48$) and seasoned (16 PP, and 15 each PSD and PT) SAT. Data were subjected to ANOVA by SPSS statistical package using the typologies of production as fixed effect. The average fatty acid composition of raw ham SAT showed high levels of MUFA (49.5%, mainly C18:1) and SFA (39.8%, mainly C16:0 and C18:0), and lower PUFA (10.7%, mainly C18:2) and, as expected, no difference was detected between the three typologies of productive destination. On the whole, during the seasoning period SAT showed an increase of MUFA (49.5 vs 53.0% for fresh and seasoned, respectively) and a decrease of PUFA (10.7 vs 8.2%). The differences among the three seasoned ham types, although statistically significant for some fatty acids, point out that, starting from homogeneous raw matter, the methods used by productive plants of the three studied PDO products do not considerably affect the final characteristics of the lipids of the obtained dry-cured hams.

Acknowledgement

The research was supported by MIUR- PRIN 2007 (Prof. D.P. Lo Fiego)

C-151

Influence of genetic type on the characteristics of intramuscular fat of pig thighs destined for the PDO production

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Italian heavy pig production uses both “traditional” breeds, whose carcass fat content has been reduced because of consumers’ demand, and commercial hybrids, whose fat depots are often low, and richer in PUFA. The increasing degree of lipid unsaturation requires a continuous monitoring to avoid undesired effects on typical products. The aim of this work was to compare the characteristics of intramuscular fat (IMF) of fresh thighs, destined for the production of PDO Italian dry-cured hams, obtained from heavy pigs of two genetic types (GT), reared in the same farm and fed a standard commercial diet based on cereals and soya-bean. Samples of *biceps femoris* from left thighs of 46 Italian Landrace x Large White pigs (TP), and 32 Goland commercial hybrids (GH) (average carcass weight 130.4 ± 11.8

kg) were analyzed for IMF, thiobarbituric acid reactive substances (TBARS) (24hrs p.m.) and fatty acid (FA) contents. Data were subjected to ANOVA by SPSS statistical package using GT and sex as fixed effects. GH showed, in comparison with TP: similar contents of IMF (2.2 and 2.7%) and TBARS (0.07 and 0.07 mg MDA/kg meat), but higher levels of PUFA (201.5 vs 157.5 mg/100 g meat, 17.9 vs 11.3%; $P < 0.01$), mainly due to higher C18:2 and C20:4; lower SFA (468.8 vs 582.3 mg/100g meat, 37.4 vs 39.5%; $P < 0.01$) mainly due to lower C18:0 and C20:0; lower MUFA (572.4 vs 730.7 mg/100g meat, 44.7 vs 49.2%; $P < 0.01$), mainly due to lower C18:1. The fatty acid composition of IMF confirms the results obtained from the covering adipose tissue of the same thighs, and agrees with many results from literature. Notwithstanding the positive lack of difference between the two genetic types for the amount of IMF and TBARS, it seems necessary a systematic check of lipid composition, to avoid undesired negative response of meat to processing due to possible oxidative phenomena during seasoning period.

Acknowledgement

The research was supported by MIUR-PRIN 2007 (Prof. D.P. Lo Fiego).

C-152

Long chain fatty acids distribution in different tissues of suckling piglets

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Aim of this work was to study the fatty acids (FA) deposition in different tissues of newborn piglets. Six piglets were collected from six different sow (LxLW) in second or third parity inseminated with semen from Landrace boar. During gestation sow were housed in individual cages fed a concentrate mixture for pregnant sows. At the end of delivery, one piglet (colostrums deprived) from each sow was exsanguinated, then eviscerated, and brain, heart, liver, kidney, skeletal muscle samples were removed. FA composition of total lipid extract of sample from each tissue was determined by gas chromatography analysis. Each FA concentration was expressed as a percentage of total FAME. Data were analyzed with ANOVA using tissue type as the main effect. The FA composition differed significantly within the different tissues (see following Table). The saturated FA (SFA) were higher in brain and muscle, while the unsaturated FA (UFA) was higher in heart where they account for 2/3 of total FA. The concentration of PUFA was higher in brain and heart. The lowest value of the $\omega 6/\omega 3$ ratio in the brain lipids is due to its high content of docosahexaenoic acid (DHA) that represents about the 97% of total $\omega 3$ FA. The high concentration of DHA in brain confirms the activity of elongase and desaturase enzymes in this tissue and the synthesis of the ex-novo long chain PUFA $\omega 3$.

Fat and fatty acid composition of different tissue of newborn piglets.

Item	Brain	Liver	Heart	Kidney	Muscle	P
SFA (% of FAME)	47.18 ^a	44.62 ^{ab}	36.86 ^c	41.92 ^b	47.54 ^a	**
MUFA(% of FAME)	26.80 ^c	38.79 ^a	31.90 ^{bc}	32.92 ^b	36.77 ^{ab}	**
PUFA(% of FAME)	26.02 ^{ab}	16.59 ^c	31.24 ^a	25.16 ^b	15.69 ^c	**
SFA/UFA	0.90 ^a	0.81 ^{ab}	0.58 ^c	0.72 ^{bc}	0.91 ^a	**
$\omega 6/\omega 3$	1.54 ^c	7.21 ^b	14.44 ^a	15.38 ^a	12.05 ^a	**

Means followed by different letters within each row are significantly different; **:P≤0.01;

C-153

Sources of variability of H, C, O, N and S stable isotope ratio in dry-cured ham

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The stable isotopes ratios (IR) of bio-elements, which depend on botanical, geographical, agronomic and climatic factors that are

transmitted from water and vegetables to animal products, are potential indicators of meat production and processing systems. The aim of the study was to ascertain the effect of the geographical origin and the production process of dry-cured ham on the variability of ²H/¹H, ¹³C/¹²C, ¹⁵N/¹⁴N, ¹⁸O/¹⁶O, ³⁴S/³²S ratios, measured by IR Mass Spectrometry after total combustion or pyrolysis. Overall, 11 IR were examined in three ham fractions: defatted dry matter (DFDM) and fat of *biceps femoris* and subcutaneous adipose tissue. The hams were made out of pigs reared in three geographical areas, where different productive factors were studied. The effect of pig genetic type and rearing system was examined on 36 hams from heavy pigs of three genotypes (Cinta Senese, Mora Romagnola and a commercial hybrid) kept on the same diet in Friuli both in stable or in a fenced woody area. The influence of feeding regime was analysed by comparing the isotope ratios of 26 *Dehesa de Extremadura* PDO hams, from heavy pigs fattened outdoor on grazed feedstuffs without ("Bellota" ham) or with ("Campo" ham) concentrate supplement. The processing influence was examined on hams from pigs reared on the same farm and diet in Emilia and seasoned for different periods following the procedures of Parma, *San Daniele* and *Toscano* PDO hams. By applying the stepwise linear discriminant analysis, 98.8% of hams were correctly assigned and 97.7% correctly cross-validated into specific types, by a model including the five IR of DFDM, together with ¹⁸O/¹⁶O of marbling fat and ¹³C/¹²C of subcutaneous fat. A full discrimination of origin was performed while, within location, autochthonous breed, feeding regime and stage of seasoning had a significant effect on IR variability.

1W

Strategies for assessing conservation priorities in sheep breeds

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The purpose of this study was to increase knowledge of the genetic diversity of local sheep populations making part of the conservation program of the Veneto Region, by analyzing 17 microsatellite markers on blood samples of individuals enrolled in the population register. A total of 695 animals of the Lamon, Alpagota, Brogna, and Foza breeds were analyzed. Sampling was performed in 55 farms for both sexes (270 male and 425 female). The software used for statistical analysis were GENETIX 5.4 GENEPOP 4.0, MOLKIN 3.0, FSTAT, PHYLIP and STRUCTURE (admixture model). On the whole, 342 alleles were detected, with an average of 20.12 alleles per locus. The most polymorphic microsatellites were *MAF214* (36 alleles) and *INRA063* (22); the lesser *TGLA53* (14). The expected heterozygosity ranged from 0.768 for Alpagota to 0.804 for Foza. The molecular coancestry within breed varied from 0.424 (for Alpagota and Brogna) to 0.433 and 0.440 (for Lamon and Foza, respectively). Private alleles with frequencies higher than 1% were only 17. Genetic diversity variations ranged from +1.080% to -1.196% for the removal of the Alpagota and the Foza breeds, respectively. Average FST was equal to 0.043. The dendrogram showed, using Reynolds distances, distinctive grouping among Foza and Brogna and for Lamon and Alpagota breeds. Structure analysis revealed separation among the local breeds with a within breed homogeneity. In conclusion good values of genetic variability were measured, mainly due to the marked differentiation among the breeds, than to their internal diversity, suggesting that Alpagota and Foza breeds seem to be the two breeds with higher priority, respect to Brogna and Lamon, in order to preserve of sheep populations biodiversity in the Veneto Region.

2W

Phylogenetic analysis of *Ovis aries* using mtDNA: D-loop vs complete sequence

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The analysis of the mitochondrial D-loop region in sheep (*Ovis aries*) revealed the presence of five haplogroups, revealing a diversity comparable with that observed in goat and cattle. However, recent studies on cattle demonstrated that the analysis of the control region alone may be inadequate for reconstructing species phylogeography. The aim of our research was to compare the information retrieved using only the mitochondrial D-loop with that obtained by analysing the complete mtDNA sequence to shed light on management and migrations that led to the formation of modern breeds in sheep. We sequenced a total of 4.1×10^5 bp of mtDNA D-loop from 932 samples belonging to 66 sheep breeds from Mediterranean and eastern Europe and 8 European mouflon (*Ovis musimon*), identifying a total of 146 polymorphic sites and 383 haplotypes. Phylogenetic analysis of these haplotypes showed four of the five haplogroups described in literature: all four were found in Turkey; C is present in Greece, Albania, Hungary and Saudi Arabia; B is the most frequent group in all countries except Turkey, where A and B are equally common. Haplogroup A frequency decreases from Middle East to mainland Europe and to western Mediterranean areas. European mouflon, representing a wild residue of the first domestic sheep that arrived in Europe, clusters in haplogroup B. Forty eight samples were selected for complete sequencing on the basis of their D-loop haplotype distance to represent most of the species variability. As expected, the control region is the most variable, although several polymorphisms in other regions of mtDNA were found. The analysis of the complete mitochondrial sequence and its comparison with D-loop may enrich data on European sheep breed diversity and establish a rationale method for assessing species genetic variability.

3W

Genetic diversity of chicken breeds native of Veneto and Toscana regions

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This study explores genetic diversity and clarify genetic relationships among four Veneto and one Tuscany native chickens populations. A total of 368 DNA samples were collected from four Veneto native breeds (Robusta Lionata, Robusta Maculata, Ermellinata di Rovigo, Pèpoi), while 76 samples were taken from five Mugellese ecotypes, being different for local distribution and phenotypes. Genotyping was performed at 19 microsatellite loci. Estimates of observed heterozygosity were higher for the Mugellese ecotypes compared to the Veneto native breeds, while parameters of expected heterozygosity were similar. Deviations from Hardy-Weinberg equilibrium were observed for all the Veneto local breeds and for three Mugellese ecotypes. For the former group an excess of homozygotes was measured, while for the lat-

ter an excess of heterozygotes. The Mugellese ecotypes prove to be good reservoirs of genetic variation, since a high loss of diversity (-18.18%) is measurable when they are excluded from analysis. This loss of diversity is mainly due to the marked differentiation from the venetian breeds (-11.24%), and only secondarily because of internal diversity (-6.94%). Within group molecular coancestry was higher for the Mugellese ecotypes compared to the venetian breeds. Reynolds and individual Ps genetic distances were calculated among groups, evidencing a marked separation between the Mugellese ecotypes and the other breeds, showing that gene flows did not occur among the two regions in the last decades.

4W

Milk production and composition in two Mediterranean goat breeds based on the alpha s1 casein locus (*CSN1S1*) genotype

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The objective of this study was to investigate the effect of three genotypes at α s1-casein locus in Girgentana (G) and Derivata di Siria (DS) goats fed ad libitum on milk yield and composition. 27 unrelated Girgentana goats and 27 unrelated Derivata di Siria goats were selected according to their *CSN1S1* genotype as follows: 9 goats homozygous (AA) for strong allele, 9 goats homozygous (FF) for weak allele and 9 goats heterozygous (AF) for each breed. The goats were uniformly characterised by strong alleles at *CSNS2* and *CSN2* loci. All the animals were housed in individual pens and goats received the same diet (5.8 MJ NE/kg DM; 15.2% CP) ad libitum consisted of a pelleted feed including 65% of alfalfa hay. The experimental period consisted of 15d for adaptation and 8d for data and samples collection. Milk samples were analysed for protein and fat by an infrared method. The nitrogen fractions and urea content were measured by FIL-IDF methods and differential pH meter, respectively. In each goat breed, data were analysed by ANOVA procedure for repeated measures. Milk production was significantly affected by α s1-casein genotype in G ($P<0.05$) and DS ($P<0.046$) breeds. In particular, DS AA goats produced more than AF and FF goats (1273.5, 1030.6 and 980.1 g/d; $P<0.05$). The same effect was observed in G breed: milk production in AA genotype was higher, compared to AF and FF (respectively: 1550, 1200 and 1050 g/d; $P<0.05$). Protein, casein and urea content were affected by genotype in both breeds. In G AA goats, the highest content of protein, casein (3.79, 2.98%) and the lowest urea content (32.14 mg/dL) were found ($P<0.05$). The same trend was observed in DS AA goats: the highest content of protein, casein (4.01, 2.80%) and

the lowest urea content (36.21 mg/dL) were detected ($P<0.05$). No effect of genotype was found on fat content.

Acknowledgement

The research was funded by MIUR, project 2007B4JBWN_003

5W

Conservation priorities for some goat breeds and populations reared in Southern Italy

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Southern Italy has a noteworthy heritage in terms of goat biodiversity but the local traditional populations are often scarcely managed: only some of them have consolidated herd books, selective breeding is absent and gene flow is almost continuous between populations reared in the same area. A genetic study was performed on a total of 174 goats belonging to six breeds and populations (31 Girgentana, 30 Argentata dell'Etna, 30 Maltese, 22 Rossa Mediterranea, 30 Messinese, 31 Capra dell'Aspromonte) reared in Sicily and Calabria. The individuals were genotyped at 20 microsatellite markers. Heterozygosity levels and allelic frequencies were assessed per locus and breed. Multilocus genotypes were also used to infer molecular coancestry coefficients and implement different conservation criteria; in view of measuring the contribution of each breed to the total diversity the methods based on gene diversity, allelic richness (A_r) and pairwise genetic distances were used. Genetic differentiation in the data set was evaluated using F-statistics tool in order to partition heterozygote deficiency into a within and an among population component. A total of 216 alleles were detected (10.8 per locus), A_r varied from 5.99 in Girgentana to 7.52 in Argentata dell'Etna. Overall F_{ST} (3%) indicated low differentiation among breeds. Priorities aimed at breeds conservation according to gene diversity, allelic richness and pairwise Reynolds' distances favoured Maltese, Messinese and Girgentana, respectively. The analysis of conservation priorities highlighted Maltese and Argentata as the most important in terms of contribution to the diversity, whilst Aspromonte the less notable among these breeds.

6W

Demographic and genetic parameters in Miniature Pinscher (*Canis familiaris*) Italian population

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The aim of this work was to investigate the genetic and demographic parameters of the Italian population of Miniature Pinscher (MP) and its evolution across generations to supply effective and objective tools for breed conservation and selection strategies. MP is a worldwide known toy breed of German origin, part of FCI group 2: Pinscher and Schnauzer - Molossoid Breeds - Swiss Mountain and Cattle Dogs. Height at withers ranges for dogs and bitches between 25 to 30 cm and the weight between 4 and 6 kg. The coat is short, dense and smooth, the colour is black and tan or red-reddish- dark red brown; MP are wonderful companion and guard dogs particularly adapted to apartment life style. Italian Kennel Club (ENCI) Stud Book official data of 25,065 subjects were analysed (12,880 females, 12,185 males); dates of birth ranged from 1965 to 2009. All the calculations were performed using ENDOG V 4.6 software. Mean maximum number of traced generations was 6.84, mean complete generations were 2.85 and mean equivalent generations 4.14. Average inbreeding coefficient (F) was 0.08 and average relatedness coefficient (AR) was 0.02. The maximum number of puppies entered was recorded in 2003 with 1111 new born MP. Alternating F values were calculated with a constant increasing between the end of the seventies and the first years of nineties (0.11, 1989), maximum calculated individual F value was 0.55. MP are characterized by a significant number of entries per year with low average inbreeding values, the coexistence of two subpopulation: 'show' and 'pet' could be supposed. Further investigation are needed to better understand the genetic asset of MP breed.

7W

Optimisation of bull epididymal spermatozoa extraction techniques: a tool for improving gene banking

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Animal gene banks development is limited by high costs of creation. The recovery and freezing of viable sperm from the epi-

didymes of slaughtered animals can be a cheap alternative for preserving male gametes. The aim of this study was to optimize the recovery of bull epididymal spermatozoa comparing the effects of two methods of extraction in combination with egg yolk extender. Testis from 23 Limousine bulls, ranging in age from 13 to 20 months, were collected at the abattoir. Spermatozoa were extracted from the epididymes using two methods: the float-up and the retrograde flushing. The average time for extraction was 16 minutes in both methods. Within each methods half of testis were processed with an egg yolk extender and the second half with an egg-yolk free extender. Sperm concentration, motility, viability and morphology were evaluated. Statistical analyses were carried out using the GLM procedure (SAS package v9.1). Methods of extraction, in combination with the presence of egg yolk in the extender, were considered as fixed effects; age of the bull and time for extraction as covariates. Sperm concentration was not significantly different using either the float-up method or the retrograde flushing (283.5±24.7 and 301.5±30.3×10⁶ cells/mL, respectively). Flushing technique was better than the float-up method in terms of sperm quality, considering total motility (78.4±3.1% vs 61.8±2.6%, P=0.0003; respectively) and viability (88.7±2.1% vs 77.6±1.7%, P=0.0003; respectively). Egg yolk influenced positively motility and morphology in the float-up method, whereas decreased viability in flushed samples. In conclusion, samples extracted by flushing method, with egg yolk, showed a better sperm quality. These data suggest the possible use of epididymal sperm collected by the flushing technique for the creation of cattle semen cryobanks.

8W

Genetic diversity and structure of Italian sheep breeds inferred by genome-wide SNP analysis: overview of the "Italian Project for Sheep Biodiversity"

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So far, genetic diversity within and among Italian sheep breeds has been investigated almost exclusively by microsatellite markers and never at national scale. Recently, high density Single Nucleotide Polymorphism (SNP) arrays have been developed for the major livestock species, including sheep. In the second half of 2010, a large national collaborative project was launched, within the context of the International Sheep Genome Consortium (ISGC), aiming at characterizing at the genomic level the main Italian sheep breeds, together with some endangered local breeds. Around 500 animals evenly distributed among 19 Italian breeds (Alpagota, Altamura, Appenninica, Bagnolese, Bergamasca, Biellese, Comisana, Delle Langhe, Fabrianese, Gentile di Puglia, Laticauda, Leccese, Massese, Pecora Nera di Arbus, Pinzirita, Sambucana, Sarda, Sopravissana, Valle del Belice) have been genotyped using a 50K SNP array. Molecular data have been pruned according to SNP minor allele frequency (MAF<0.01), frequency of missing genotypes (Geno>0.1) and linkage disequilibrium ($r^2 > 0.5$) using the PLINK software package. The subsequent analysis of the massive data generated by genome-wide SNP genotyping will pinpoint the genetic distinctiveness and levels of admixture of Italian breeds, thus unraveling new aspects of the breed formation process. In addition, comparative analysis of conserved haplotypes will identify genomic segments under selection pressure, highlighting interesting genomic regions. Data analysis will also be useful to select SNP loci suitable for parentage and identity DNA test in Italian breeds.

9W

Differential expression of sarcoplasmic proteins in meat of lambs reared in Continental Southern Italy

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The proteomic analysis defines the identity, the structure and the relative abundance of proteins in a given type of cell and in a specific set of conditions.

The analysis of protein profile was carried out to identify possible expression differences of muscular proteins between sheep breeds and use these peculiar profiles as trace back biomarkers. The analyses were carried out on a total of 98 samples of *longissimus dorsi* and of leg muscles pools from 49 ovine belonging to five breeds: Altamura (N=9), Bagnolese (N=10), Gentile di Puglia (N=10), Laticauda (N=10) and Leccese (N=10). The protein profile was investigated using analytical proteomic procedures: 2DGE and MALDI-TOF/MS. The two-dimensional maps were compared by image analysis using Image Master 2D-Platinum to define the position and relative intensity (volume %) of every single spot. In the range of our observation, image analysis highlighted 15 spots common to all samples, identified as: Adenilate kinase, DJ-1, Enolase (2 spot), Hemoglobin (Hb), Myoglobin (Myb) (2 spot), Phosphatidylethanolamine binding protein (PEBP), Superoxide dismutase, Triosephosphate isomerase (TPI) (5 spot) and Ubiquitin. With the exception of Enolase, the expression (volume %) of common spots differed between breeds, within the muscle. There were significant ($P < 0.05$) differences of expression between breeds for 5 spots: Enolase, Myb and TPI (2 spots) for the 'Altamura'; Ubiquitin for the 'Gentile di Puglia' and 'Leccese'. It was furthermore detected a different expression of some proteins between two breeding 'bioterritories': (i) higher expression of Myb and of some TPI spots in the breeds reared in the 'Pugliese' 'bioterritory'; (ii) higher expression of DJ-1, Hb, PEBP and Ubiquitin proteins in the breeds reared in the 'Sannita' 'bioterritory'. These differences might probably be ascribed to environmental effects. Thus obtained results suggest a possible use of the proteomic approach to define physiological peculiarity in specific bioterritories; this may allow to establish a link between ovine meat and the territory that can be used to safeguard local productions.

10W

Ancient autochthonous genetic type Casertana pig: influence of slaughter age on fatty acid composition of subcutaneous adipose tissue

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Fatty acid profile of lipids in animal origin products (AOP) for human food regimen plays an important role for its effects on consumer health. Over time 'lard' has played an useful function in the composition of a ration for human diets. A reevaluation of biochemical effects of 'lard', as a source of biomolecules with nutritional and extranutritional properties, is in progress. The Autochthonous Genetic Types, especially ancient types (AAGT), can be considered in future a particularly useful source of this AOP. 'Casertana' pig, known since ancient times, deserves AAGT title. The study was carried out on 'fresh lard' taken at the level of 4th ÷ 6th lumbar vertebra from 50 'Casertana' pigs (21 castrated males and 29 females) bred at Experimental Station of ConSDABI (Circello, Benevento). The acidic fraction was extracted by Folch method and analyzed, as methyl esteres, by gas-chromatography. The acidic fraction considered consists of: palmitic, palmitoleic, margaric, eptadecenoic, stearic, oleic, C18:1 cis,12, linoleic, γ -linolenic and linolenic fatty acid and CLA, the more representative is C18:2 cis9, trans11 and the other 6 isomers were detected in traces. The effect of sex, year and month of slaughtering (covariates), age and refrigerated carcass weight was always significant ($P < 0.03 \div 0.0002$) on all fatty acids as well as on 'atherogenic' and 'thrombogenic' indices. Therefore, in evaluating the utilization of 'fresh lard' from a health point of view for human food, it is possible to define the models of optimization of lipidic profile, especially in an innovative vision of 'molecular' gastronomy.

11W

Molecular characterization of swine ancient autochthonous genetic types by analysis of microsatellite markers

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Genetic characterization is essential in a plan of safeguard of indigenous breeds. Knowledge of the level of genetic variability of Casertana (CT), Calabrese (CA) and Macchiaiola (MA) pig Ancient Autochthonous Genetic types (AAGTs) by genotyping of 18 microsatellite markers helps in planning protection activities of such AAGTs (monitoring, conservation, product valorization,

reproduction, etc.). The genotyping of 155 pigs [CT (N = 73), CA (N=50) and MA (N=32)] was carried out according to the method reported by Matassino et al. (2006). The statistical analyses were performed using software: GENEPOP (v. 3.4), MOLKIN (v.2.0) and STRUCTURE (version 2.2). When compared to MA and CT, CA had a lower value of the mean observed number of alleles [3.39 vs 4.89 vs 5.11 ($P=0.0015$; $P=0.0012$)]. The mean values of observed heterozygosity was significantly different in the 'CA vs CT' comparison (0.445 vs 0.608; $P = 0.0336$) whereas not significant in the 'CA vs MA' comparison (0.445 vs 0.566; $P=0.0918$). These results suggested the presence of a lower genetic diversity in CA, as confirmed by values of molecular inbreeding coefficient (0.551 vs 0.492 vs 0.392) and molecular coancestry (0.479 vs 0.375 vs 0.355). The F_{st} value showed a significant degree of diversification ($P < 0.01$) among the three genetic types (0.183, CT vs MA; 0.212, CT vs CA; 0.204, CA vs MA) suggesting the absence of recent gene flow among the breeds. The results of population assignment test confirmed the presence of this differentiation, since all subjects analyzed were assigned to the AAGT of origin, except for Calabrese pigs, which seemed to be divided into two subpopulations. The results suggest the usefulness of a plan of protection for the three AAGTs studied.

12W

Molecular characterization of a donkey population of Lazio. Preliminary results

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In Italy there is a considerable heritage of genetic diversity in serious danger that needs to be safeguarded. It is being in place a gradual disappearance of autochthonous breeds accelerated, among the other things, by farming abandonment in marginal areas. Genetic typification leads to the establishment of STR marker dataset; which represents a useful tool for performing population assignment test (Structure 2.2v). The donkey population typified over the years is used in a variety of farm works and, therefore, it is an integral part of the bioterritory of Lazio. 58 subjects (20 ♂ and 38 ♀) were typified at 11 microsatellite loci according to the procedure of Matassino *et al.* (2009). It should be noted that of those subjects, 50 were bred in the bioterritory of Viterbo (18 ♂ and 32 ♀) and 8 (2 ♂ and 6 ♀) in that of Frosinone. The results showed that: (i) mean number of observed alleles (Na): 5.82 ± 2.86 , CV%=49; (ii) mean effective number of alleles (Ne): 3.01 ± 1.56 , CV%=52; (iii) mean value of heterozygosity

ity: $H_o=0.534\pm 0.168$, $CV\%=31$; $H_e=0.597\pm 0.184$, $CV\%=31$; (iv) loci in significant disequilibrium from Hardy-Weinberg law: 2 ($P<0.05$), the disequilibrium was ascribable to a defect of heterozygote genotypes. Moreover, the genetic variation was higher in females than in males; this was also confirmed by lower values of molecular coancestry (0.434 vs 0.442) and inbreeding (0.447 vs 0.490). The typification of both a wider number of subjects and other donkey breeds with structure method is ongoing for a comparison among populations with a view to eventually include this population in the Zootechnical Book of horse and ass breeds at limited diffusion.

13W

'Podolian' cattle: quanti-qualitative production of the milk in cows machine milked

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The purpose of the work was to detect the milk yield and milk composition in Autochthonous Italian Grey Cattle (ex Podolian) machine milked. The Podolian breed derived from *Bos primigenius primigenius* (Aurochs) of the Near East with possible introgression from indigenous Aurochs of Middle Southern Italy. Milk production was detected in a population of 97 cows of Masseria Colombo farm. The milking technique adopted contemplates that, for around 8 months, after the machine milking of the morning, the calf remains with its mother for about 7-8 h. Therefore, the milk yield is referred to a single milking/day. The first recording data was carried out in May 2010 and the last one in January 2011. The aptitude to machine milking was good. As expected, the individual milk yield, on average, increased with the parity order reaching in the third order: kg 213 ± 50 at 30 days, kg 421 ± 94 at 2 months, kg 609 ± 116 at 3 months and kg 1215 ± 186 at 7 months ($P<0.05-0.001$) with extreme values of minimum kg 1011 and maximum kg 1595 at this latter age. In the milk at 210 days, independently of the parity order, the percentage was, on average, 4.10 for fat, 3.90 for protein, 3.05 for casein (78% of the total protein) and 4.97 for lactose. In almost all controls somatic cells were lower than 300,000. In conclusion, the Podolian genetic type subjected to machine milking showed: (i) a milk yield of remarkable interest; (ii) a composition particularly suitable for cheese-making in order to obtain high quality dairy products, as already widely known. Finally the cows showed a behaviour that we can define of 'good' answer or adaptability to the machine milking.

14W

Genetic characterization of Italian turkey breeds by microsatellites markers: a preliminary study

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The process of domestication and selection of the wild turkey (*Meleagris gallopavo*) has led to a number of breeds and populations showing many genetic characteristics and variants. Turkey biodiversity is a genetic resource at risk considering the general loss of genetic variability that characterizes poultry business worldwide. The studies of traditional turkey breeds characteristics are scarce and the analysis of their genetic make-up play a fundamental role in their conservation programmes. A total of 30 birds were analysed: Brianzolo (n=11), Nero d'Italia (n=4), Colli Euganei (n=12), Narragansett (n=3), unrelated in second generation. Genomic DNA was extracted from blood samples using classic procedures. All birds were genotyped at 31 microsatellite loci (FAO panel) isolated from domestic chicken (*Gallus gallus*) evaluating the cross amplification too. Eight multiplex PCR were performed. The PCR products were separated by electrophoresis in 4.2% denaturing polyacrylamide gels on ABI Prism 377 DNA Sequencer equipped with Genescan and Genotyper software (Applied Biosystems). Allele frequencies and deviation from Hardy-Weinberg equilibrium (P-value) at microsatellite loci were calculated using the GENEPOP statistic package. The heterozygosity (H) and the Polymorphism Information Content (PIC) were recorded. Factorial analysis three-dimensional distribution was carried out using the GENETIX program. The results of this preliminary study show the genetic variance existing in the studied populations; these three breeds are examples of Italian turkeys population particularly adapted to welfare-friendly rearing systems and niche production.

15W

Genomewide scan of Piedmontese cattle for calving ease

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Calving is affected by calf morphology and by the dam characteristics. The presence of biological effects due to both dam and calf is described by two different traits, calving ease, which is the ability to generate dams with good physiological predisposition to calving, and birth ease, which is the ability to generate calf that are easily born. The aim of this study is to identify regions of cattle genome possibly affecting calving ease in the Piedmontese breed. The studied population was formed by 393 bulls of Piedmontese breed born from artificial fecundation and tested for their birth ease and for the calving ease of their daughters. A 54,000 SNP marker panel was used to investigate the genome. Markers and bulls with more than 5% of missing data or SNPs with a minimum allele frequency under 2.5% or not in Hardy-Weinberg Equilibrium were ignored. A genome wide scan was performed, using Estimated Breeding Values as continuous variables, using the Benjamini and Hochberg method to account for false positives. Several significant SNPs associated to calving ease were detected. The strongest signal was detected on chromosome 6, in the region comprised between 37 and 39 Mbases, where several SNPs had extremely low P-values. Studies in the literature point to the existence of a QTL for these traits on chromosome 6. Two genes are located in the region containing the significant SNPs, *LAP3* and *NCAPG*. The first, leucine aminopeptidase 3 is responsible of oxytocin (a hormone of fundamental importance in female reproduction) hydrolysis. The second gene is the non-SMC condensin I complex, subunit G; recently it has been shown that a polymorphism in this gene (1326T>G) is significantly associated to fetal growth. In conclusion, this study allowed the identification of several SNPs related to calving ease and that can be considered possible selection targets and obtained results are well supported by existing literature.

16W

Selection signatures around candidate genes in Italian Brown cattle breed

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Italian Brown breed is largely exploited in the production of many typical and traditional dairy products. Thus, improvement of selection methods is of economic relevance and a deeper understanding of genetic mechanism regulating milk production is of general scientific interest. We applied a functional candidate gene approach to search for genes associated to Daughter Yield Deviations (DYDs) for milk, fat and protein yield. We tested for association 59 SNPs in 29 genes genotyping them on 561 Italian Brown bulls. A total of 29 SNPs in 23 candidate genes and 473 Italian Brown bulls were retained after the filtering process. Association between each SNPs, SNP haplotypes and each of the three traits was tested by a mixed model approach. Significant association between trait and candidate genes was detected for 7 SNPs. Then, we tried to detect pre-existent selection pressure in the genomic regions where association was present by calculating fixation indexes. A high density SNP marker panel (54,000 SNPs) was used to investigate the genome of Italian Brown bulls and for two populations formed by 488 Italian Simmenthal and 888 Italian Holstein Friesian bulls. Overall, heterozygosity values in all breeds observed were usually lower than expected values. Chromosome-wise analysis showed that fixation index values near the 7 SNPs showed relevant genetic diversity among the three breeds, indicating differentiation and the existence of selective sweeps of variable extent.

17W

Functional characterization of promoters in porcine genes affecting economical traits

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Meat quality traits are economically important in swine; however, the underlying genetic nature is very complex. Therefore, an improved pork production strongly depends on identifying and studying how genetic variations contribute to modulate gene expression. Promoters are key regions in gene modulation as they harbour several motifs binding to transcription regulatory factors. Therefore, polymorphisms in these regions are likely to deeply affect RNA levels and consequently protein synthesis. In this study, we report the identification of single nucleotide polymorphisms (SNPs) in promoter regions of candidate genes involved in development, cellular differentiation and muscle growth of muscle. The functional activity of promoter haplotypes was analysed *in vitro* with the Dual-Luciferase Reporter Assay System (Promega). Genomic DNA was isolated from muscle tissue of 10 animals belonging to Large White, a cosmopolitan and industrial breed that excel in lean meat production and 10 belonging to Casertana, an Italian breed adapted to free-ranging and with the characteristic of accumulating fat. Each promoter was amplified with flanking primers, designed on sequences available at NCBI. We identified SNPs in the promoters of the Myogenic Factor gene (*myf*), of the Myogenic Differentiation gene (*myoD1*), and of the Myostatin gene (*gdf8*). The promoter regions, harbouring only a single base pair difference, were cloned directly into the pGL4.17 vector, upstream and in frame to the luciferase (*luc+*) gene. These constructs, harbouring allelic variants, were transfected into cultured mammalian cells. Here we report the transient transfections performed with *myf*, *myoD1* and *gdf8* constructs in the CHO and C2C12 cell lines. Preliminary results of the *in vitro* analysis efficiency of promoter variants, seem to prove differences between the two haplotypes of all tested genes.

18W

Analysis of 12 candidate genes for meat production in the Piemontese cattle breed

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To date a great number of candidate genes for meat production have been suggested, based on their position and function. We selected from literature 12 genes possibly related to growth and development (*GH*, *GHR*, *GDF8*, *GHRL*, *IGF2*, *LEP*, *LEPR*, *MYF5*, *NPY*, *POMC*, *UCP2* and *UCP3*) and studied their variability in the Piemontese breed, focusing the attention on a total of 20 already described SNPs. The analyses were carried out on 227 Piemontese male calves preselected for the performance test. Genotyping was performed by Kbioscience. Allele frequencies were estimated by simple counting; HW equilibrium and linkage disequilibrium for SNPs in the same gene were estimated using the FSTAT software. The survey revealed that the Piemontese breed is monomorphic at six out of the 20 SNPs: g.9371C>T in

GHR (U15731), g.844T>A, g.409G>C (AJ438578), g.365A>C in *GDF8* (AY725215), g.358C>T in *GH* (AY445811), g.284A>G in *NPY* (AY491054). As the variability described for these SNPs in different breeds is in general very low, the absence of polymorphism in the Piemontese can be interpreted as a characteristic of the breed rather than as the effect of selection against unfavourable alleles. As regards the polymorphic SNPs, a quite different variability was observed, with the frequency of the minor allele ranging from 0.07 (g.115C>T in *LEPR*, AJ580801) to 0.46 (g.149G>A in *GHR*, AF126288). For all the SNPs the genotype distributions were in HW equilibrium. A significant ($P<0.05$) linkage disequilibrium was observed for the pairs g.149G>A-g.300G>A in *GHR* (AY643807), g.305C>T (AY138588)-g.1759C>G in *LEP* (AB070368), g.666A>G-g.3032T>C in *NPY* (AY491054). The observed variability allows the study of the relationships with meat production in the Piemontese breed.

19W

Analysis of genome wide polymorphisms in the Nero Siciliano pig breed

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Nero Siciliano is an autochthonous pig breed reared in the internal areas of Sicily island (Italy). Its breed herd book was established in 2001 and, at present, the animals of this breed are reared in more than 100 farms that account, on the whole, less than 1000 gilts. The animals are usually completely black with a dorsal stripe but a few present white portions mainly in the face or in the fore legs. Due to the recent increase of the market for local and typical products, the Nero Siciliano breed has been rediscovered by farmers who have established a consortium with the aim to protect, valorise and characterise the products obtained from this pig breed. Genetic conservation programme and DNA based authentication protocols of meat of this breed rely on genetic variability that should be monitored and evaluated comparing information available for other breeds and populations. In this study we genotyped the Illumina PorcineSNP60K chip in 48 Nero Siciliano pigs chosen based on phenotypic differences and reared in different farms in order to represent the genetic variability presents within this breed. Genotyping results have been compared with data obtained by other studies with the same genotyping platform in Italian Large White pigs. The results indicated that variability in Nero Siciliano breed is comparable to that observed in Italian Large White pigs. However, other studies, including more animals, should be considered to evidence putative private alleles that might be useful for authentication of Nero Siciliano meat.

20W

Analysis of association between a *MUC4* gene polymorphism and carcass and performance traits in Italian heavy pigs

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Several studies have investigated the porcine mucin 4 (*MUC4*) gene as candidate receptor for *Escherichia coli* F4ab/F4ac that is responsible for resistance/susceptibility to infection with enterotoxigenic *E. coli* causing diarrhoea and mortality in neonatal and newly weaned piglets. In particular, a polymorphism in intron 7 (g.8227G>C) of *MUC4* is associated with resistance, even if occasionally not in complete linkage disequilibrium with the causative unknown mutation. According to literature data, surprisingly the two *MUC4* marker alleles have balanced allele frequencies in some pig populations, despite its strong association with pathogen resistance/susceptibility. In this study we analysed the *MUC4* g.8227G>C polymorphism in one performance tested Italian Large White population consisting in extreme and divergent pigs for average daily gain (ADG) estimated breeding value (EBV; 180 with positive and 180 with negative values) and in one performance tested Italian Duroc population made by 208 animals not selected by any phenotypic or genotypic criteria. Differences of allele frequencies between the two extreme divergent groups of Italian Large White pigs were statistically significant ($P < 0.001$). The susceptible allele was more frequent in the group of pigs with positive ADG EBV. No significant association with any carcass and performance trait was observed in the other population. These results might indicate that one of the reasons that prevents the elimination of the susceptible allele in the Italian Large White population could be its positive effect on post-weaning growth rate.

21W

Genetic variability of Calabrese donkeys by microsatellite analysis

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The aim of this study was to characterize the genetic pool of a Calabrese donkey population and to evaluate genetic diversity comparing this population with other eight Italian autochthonous donkey breeds (Amiatino, Asinara, Grigio Siciliano, Martinafranca, Pantesco, Ragusano, Romagnolo and Sardo). A total of 40 blood samples of Calabrese donkeys were collected in different farms in the Aspromonte area (Calabria). Genomic DNA was amplified for 12 microsatellite loci (*HTG10*, *VHL20*, *HTG7*, *HTG4*, *AHT5*, *AHT4*, *HMS3*, *HMS6*, *HMS7*, *HMS2*, *HTG6* and *ASB17*) in two multiplex PCR reactions. Genotyping data obtained for the Calabrese donkey population were compared to the genotyping results obtained for the other donkey breeds. Data were analysed using different software (MICROSATELLITES ANALYSER, FSTAT and MICROSAT) to calculate basic population parameters, including genetic distances among breeds/populations. Estimated genetic distances showed the lowest values for Romagnolo, Ragusano and Grigio Siciliano donkeys (0.025, 0.026, 0.027 respectively). GENETIX and STRUCTURE software were used to evidence potential population structures among the Calabrese population and the other considered breeds. The results indicated that Calabrese donkeys are not genetically homogeneous and do not clearly separate as an independent cluster.

22W

Single strand conformation polymorphism analysis in the Sarda breed sheep *POUIF1* gene

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The ovine *POUIF1* gene, localized in chromosome 1, contains 6 exons and 5 introns and in several mammals species its association with different productive traits has been shown. The aim of the study was to study the *POUIF1* gene nucleotide sequence to highlight possible polymorphisms and, if present, their relationships with milk productive traits in Sarda sheep breed. The study has been conducted on 140 Sarda lactating ewes, in their third to fifth lactation. A blood sample was collected from each ewe for DNA extraction. Genomic DNA (100 ng) has been used for the amplification of the exons 1, 2, 3 and 6. For each exon, 10 amplicons random selected were sequenced and compared with those present in Data bases. Then all the PCR products were denatured and analyzed using Single Strand Conformation Polymorphism (SSCP) method. SSCP analysis of exons 1, 2 and 3 did not shown any variation. Instead a G to an A substitution in the 3' UTR region of exon 6 was found in one animal. The *POUIF1* gene sequence showed a high variability in the "Churra da terra Quente" sheep, a recent Portuguese breed with a great genetic and productive variability. This productive variability in the daily milk production, that ranges from 0.25 to 1.5 l, is not associated with management differences but may be linked to a high genetic variability. However no relationship between *POUIF1* polymorphism and milk productive traits was found in this breed. Conversely in Sarda sheep the individual milk production levels are very comparable, due to genetic selection for increased milk yield. This fact could have led to an indirect selection of the *POUIF1* gene causing its lack of variability. However, it would be necessary to extend the study to the remaining exons and to other gene fragments (introns, promoter) to expanding knowledge about this gene in Sarda breed sheep.

Acknowledgement

This work was supported by grant from Fondazione Banco di Sardegna

23W

Microsatellite based genetic variation in the Siciliano horse population

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Sicily detains an ancient tradition of horse breeding, but nowadays only the 8% of the Sicilian equine patrimony is represented by autochthonous horses. The ancient Siciliano horse is an heterogeneous population that might be considered the most authentic Sicilian equine genetic heritage, linked to the Persano breed reared in Sicily in the "Borbon Real Casa di Ficuzza" until 1834. Today, the population amounts to about 400 heads reared in small nucleus (1-4 animals) in extensive systems. The aim of this work was to assess the genetic variation and to provide tools for conservation strategies for this autochthonous population. Genetic analysis was performed on a representative sample of Siciliano horses (138 animals) reared all over Sicily. A set of 16 microsatellite markers, located on 11 chromosomes, was implemented in the characterization analysis by means of the automatic sequencer ABI PRISM 377 equipped with GeneScan and Genotyper softwares. Molecular data were analysed using Genalex, Genepop, MolKin and Fstat softwares. A total of 121 alleles were detected (from 4 to 10 per locus; 7.56 on average). The mean observed (Hoss) and expected (Hexp) heterozygosity were 0.712 ± 0.021 and 0.750 ± 0.020 , respectively. The population was in genetic equilibrium and Fis value across loci was close to zero (0.050) so that random mating in this population can be assumed. Genetic diversity measures revealed sufficient genetic variability in the Siciliano horse; mean number of alleles, expected heterozygosity (Hexp) and Fis in this population were comparable to the values reported for other horse breeds in Europe. Comparison with the other two Sicilian native horse population, namely Sanfratellano (61) and Sicilian Oriental Purebreed (50), was also carried out and different methods for establishing conservation priorities are being discussed.

24W

Angiotensin-I converting enzyme inhibitory activity of cow, buffalo and donkey milk protein hydrolysates

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The renin-angiotensin-aldosterone system is a target for blood pressure control. Angiotensin-I converting enzyme (ACE) plays a central role in the regulation of blood pressure. Milk proteins, both caseins and whey proteins, are a rich source of ACE inhibitor peptides. The aim of the research was investigated the effects of cow, buffalo and donkey caseins and defatted milk hydrolysates on ACE activity *in vitro* to relate the activity of ACE inhibitor peptides with interspecies differences. Total caseins were obtained following the method of Zittle (1966). *In vitro* human gastrointestinal digestion of caseins and defatted milk was performed with pepsin, trypsin and chymotrypsin. The ACE activity inhibitory (ACEi) effect of casein and defatted milk hydrolysates was measured by spectrophotometric assay according to Cushman and Cheung (1971). The ACEi was expressed as IC₅₀ value (concentration of inhibitor mediating a 50% inhibition of ACE activity). The results were expressed as means \pm SEM, and statistical significance was tested with ANOVA. Casein and defatted milk hydrolysates, of all species considered, had ACEi effect. In each species, defatted milk hydrolysates showed higher IC₅₀ values than casein hydrolysates (P<0.01). In contrast, there were not significant differences between species both for defatted milk or casein hydrolysates. Casein hydrolysates were potent ACE inhibitors (IC₅₀ values were 19.03, 30.71 and 32.30 μ g/mL for bovine, donkey and buffalo, respectively). Finally, peptides derived from casein and defatted milk have an effect on ACE activity *in vitro*. *In vivo* studies are in progress to confirm this effect.

25W

Influence of sonication on milk sanitization and sensorial traits

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Among new technologies used to improve milk sanitization and shelf life, the treatment with ultrasounds showed positive effects on bacterial inactivation. The aim of this study was to evaluate the influence of different sonication parameters on *E. coli* inactivation and on milk sensory properties. An ultrasonic processor was used at different combinations of amplitude of ultrasonic wave (A: 70 vs 100%), cycle (C: 0.5 vs 1) and treatment time (T: 100 vs 240 s) on UHT milk inoculated with *E. coli* (ATCC 11775) cells (10⁵ CFU/mL). Before and after each treatment samples were plated in Plate Count Agar (PCA) and in Violet Red Bile Agar (VRB). All treatments were conducted in triplicate. To evaluate the effect of ultrasounds at different values of A (0-100%), C (0.5-1) and T (50-100s) on whole milk sensory properties, a triangle sensory test was carried out. Data were submitted to ANOVA according to a linear model. The increments of A, C and T, resulted in a higher *E. coli* inactivation in both PCA and VRB. The values of CFU/mL log reduction were (A: 0.50 vs 0.67, P<0.01), (C: 0.30 vs 0.89, P<0.001) and (T: 1.01 vs 1.92, P<0.001). Depending on intensity and duration of sonication, the taste led to discriminate between treatments at 75 and 100% whereas the smell allowed to identify treated and untreated milk at 0, 66 and 100% with increasing intensity and duration. Treated milk resulted unpleasant if compared to control milk. The rating values were significantly different between treatments (P<0.01) and ranged between 4.5 of control milk to 2.25 of milk sonicated at amplitude 100%, cycle 1 for 100s, where a scale between 1 (unfit for consumption) and 5 (very good flavour) was used. Off-flavours in sonicated milk probably resulted from creation of organic sulfur compounds that arise during the decomposition of reactive protein sulfhydryl groups associated with the amino acids, methionine and cysteine. Sonication resulted effective against bacteria but some corrections have to be found to limit sensory degradation.

26W

Effect of herd size on physicochemical characteristics, hygienic-sanitary quality and coagulation properties of milk for Parmigiano-Reggiano cheese

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The effect of herd size (n. reared cows) on milk quality parameters for Parmigiano-Reggiano cheesemaking was studied.

The survey was carried out on 38,928 herd milk samples collected from 594 herds, throughout 10 years. On each milk sample, the

milk quality payment parameters were determined. Data were processed by ANOVA, using as fixed factors the herd size (class 1 = <30 cows; class 2 = 30÷60; class 3 = 61÷100; class 4 = 101÷200; class 5 = >200), the pedoclimatic zone (hill, plain), the season (winter, spring, summer, autumn); the significance of the differences was tested by LSD control. The lactodynamograms were grouped in 3 classes (optimal, types A, B, C, EA, EB, EC; discrete, E, D, EF, DD; anomalous, FE, F, FF) and the frequencies were analysed by chi-square method. Increasing herd size was positively correlated with milk production (kg milk/cow/lactation) (from 7,067 class 1, to 8,769 class 5), milk somatic cell content (from 264,000 class 1, to 323,000 cell/mL class 5), and clostridia spores (from 78 class 1, to 177 spores/L class 5). Conversely, herd size was negatively correlated with milk fat (from 3.68 class 1, to 3.42 g/100g class 5), total bacterial count (from 98,000 class 1, to 47,000 CFU/mL class 5) and coliforms (from 1,903 class 1, to 718 CFU/mL class 5). Finally, the milk produced in class 1 herds showed a high frequency of anomalous lactodynamographic types (1.1%) than class 5 herds (0.5%). The management conditions differ among herd according to the number of reared cows. These difference were able to affect the main quality parameters of milk for Parmigiano-Reggiano cheesemaking.

27W

Fatty acid profile and nutritional index of goat milk were affected by forage species

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The objective of this study was to evaluate the effect of herbage species on the fatty acid profile and the Health Promoting Index (HPI) in goat milk. Forty Derivata di Siria goats homogeneous for milk production, days of lactation and body condition score were allotted to eight groups and fed with the following plant species: *Avena sativa* (AS), *Lolium perenne* (LP), *Triticosecale* (TG), *Hordeum vulgare* var. Federal (HV), *Medicago sativa* (MS), *Trifolium incarnatum* (TI), *Vicia sativa* (VC), *Lotus corniculatus* (LC). All species were used in pureness. Each experimental period lasted 11d and consisted of 8d for adaptation and 3d for milk sampling during which the goats received ad libitum the fresh forage cut daily. Plant species affected ($P<0.01$) classes (SFA, MUFA, PUFA), single FAs (ALA, CLA) and nutritional index (HPI). SFAs content was significantly higher in LP than in milk from AS, HV, TG species. MUFA values significantly decreased from TG and HV (35.3 and 34.2) to LC and MS (29.5, 29.1) and reached the lowest values in milk from LP (24.7) diet. PUFA content was higher

($P<0.01$) in LC (8.35) and lower in LP (6.09) milk. The CLA content of milk from goats fed AS, LC, HV and TI (0.93, 0.92, 0.97 and 0.95) diets was higher than MS and VC (0.67 and 0.62) diets. The proportion of ALA was maximum with the VC (1.44) and minimum with LC and MS (1.17 and 1.06) diets. In the other groups ALA content ranged from 0.67 to 0.84. We found a significant effect of forage species on HPI. In particular, this index was significantly higher in TG (0.77) than LP (0.43) milk. This study showed that the forage species affected markedly the fatty acid profile and the nutritional index of milk.

28W

Effects of indigenous enzymes on proteolysis in goat milk

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Caprine milk is of great importance in many countries for its nutritional and functional properties. Proteolytic activity in fresh raw milk is attributed to indigenous enzymes and microbial enzymes. Most studies on indigenous milk enzymes have been carried out on bovine milk and less information is available for other species. This study was undertaken to evaluate the activity of the main indigenous proteolytic enzymes in caprine milk and their relevance to hydrolysis of casein fractions. Bulk samples of Garganica caprine milk were collected on three occasions from ten dairy farms located in the Gargano area in southern Italy. Bulk milk was analyzed for chemical composition, plasmin, cathepsin D, and elastase activities and renneting parameters. Bulk milk was pasteurized (63°C for 30 min), then lyophilized and stored at 4 °C. Lyophilized milk was reconstituted to 9% (w/v) total solids and then incubated without inhibitors as control, with aprotinin (inhibitor of serine proteases), pepstatin (inhibitor of aspartic proteases), or a mix of these inhibitors, at 37°C for 7 d. Milk with or without inhibitors was sampled after 0, 1, 3, 5, and 7 d of incubation for sodium dodecyl sulphate polyacrylamide gel electrophoresis. The gels were analyzed for bands identification. Determination of enzymatic activities in milk evidenced higher level of plasmin activity than that of cathepsin D and elastase in bulk milk. The serine proteases, i.e., plasmin and elastase, were relevant to the hydrolysis of β -casein and were partly responsible for hydrolysis of α -casein whereas aspartic proteases, such as cathepsin D, operated a limited proteolysis in milk.

29W

Transfer of milk fatty acids to Pecorino Siciliano cheese

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In previous studies it was observed that milk fatty acid (FA) composition is mainly affected by diet, and when ruminants graze pasture the percentages of polyunsaturated FA and conjugated linolenic acid (CLA) are higher than in milk from preserved forage. The maintenance in the cheese of the beneficial FA profile of milk from grazing ruminants is advisable. Little number of study exists concerning the effect of milk processing conditions on cheese FA profile, but no study was done with regard to Pecorino Siciliano cheese. The aim of this study was to evaluate the transfer of FA from ewes milk to cheese manufactured according to the Pecorino Siciliano technology, in order to verify if the cheesemaking process could modify the FA profile of cheese, as yet observed by other authors for CLA and C18:1 isomers. Bulk milk from Comisana ewes grazing a spring pasture was processed six times during three consecutive weeks. For each cheesemaking, the 48-h bulk milk was divided equally, one part was processed as raw milk and the other one was pasteurized (72°C for 20 s) and inoculated with a starter culture. FA transfer from milk to cheese did not seem to be greatly affected by cheesemaking technology also when milk was treated by pasteurization. However, some significant differences were recorded for C11:0 ($P=0.0434$) and C14:1c9 ($P=0.0132$) that resulted higher in cheese than in milk. Also some isomers of CLA, such as C18:2 t-9, c-11 and CLA C18:2 t-11, t-13, resulted higher in cheese than in milk ($P=0.058$ and $P=0.0058$, respectively), but the difference reached the significance only between milk and cheese from raw milk, probably as consequence of a different activity between natural microflora and added microorganisms. This trial showed that during the cheesemaking the totality of milk FA are transferred into the cheese even when the milk is processed after a thermal treatment. Nevertheless, some variations had been observed with regard to CLA isomers, in line with other findings on sheep cheese produced in other Italian regions.

30W

Field study on milk fat and protein ratio reversion in dairy cattle

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Milk fat and protein ratio reversion (MFPR) has been related with insufficient dietary fibre quantity and quality, excess of concentrates and sorting by the cows. The aim of the present work was to study the effects of nutrition and season on the occurrence of MFPR. Three dairy herds (A,B,C) similar for dimension, parity and days in milk, producing milk for direct consumption, fed corn silage based rations were observed over a period of 12 months. Herds were fed similar diet but differed for nutritional management during summer period. During this period herds were fed 3 different levels of fat, NSC and NDF: herd A received a diet containing 3.52% of fat while herds B and C were fed 4.26 and 5.06% dietary fat; NSC levels were 41.1, 40.8 and 32.9% while NDF levels were 33.7, 33.0 and 35.8% for herd A, B and C respectively. Representative samples of total mixed rations were collected monthly and diet composition and digestibility variations were monitored. Individual milk fat and protein content data of the cows were obtained from the Associazione Provinciale Allevatori monthly reports and MFPR calculated considering as inverted cows with milk fat lower of more than 0.3% than protein. MFPR incidence showed high variability between herds. On average, the minimum incidence of MFPR was registered during winter (15.6%) while this phenomenon showed the maximum incidence in summer/early autumn (29.4%). In general, a significative ($P<0.01$), negative (-0.492) correlation was found between NDF level and MFPR incidence. Compared to the yearly trend of the herds A and B, herd C did not show an increase of the incidence of fat:protein reversion during summer. This is probably related to the higher NDF and higher dietary fat administered to the herd C during this period with respect to herd A and B, whose rations were also higher for NSC content.

31W

Whole flaxseed improves milk production and fatty acid profile of lactating ewes during heat stress

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Milk yield and composition were studied after provision of shade and flaxseed supplementation in lactating ewes during summer. The experiment was conducted during the summer of 2007 and involved 40 Sarda ewes divided into 4 groups. A 2 x 2 factorial design was used, with ewes being supplemented with whole flaxseed (F) or not (C), and exposed (EXP) or protected from solar radiation (PRO) for each dietary treatment. Milk samples from each ewe were collected every week, and analyzed for pH, total protein, casein, fat, and lactose content, somatic cell count (SCC), and renneting parameters (clotting time, rate of clot formation, and clot firmness after 30 min). Milk samples were also analyzed for milk fatty acids. Flaxseed supplementation improved milk yield, fat, protein, and casein yields and increased fat and lactose content of milk. Fat supplementation also reduced milk SCC. A

decrease of saturated fatty acids (SFA) and an increase of C18:1 trans-11 and C18:2 cis-9, trans-11 emerged in milk from flaxseed supplemented ewes. Flaxseed supplementation increased the monounsaturated fatty acids content (MUFA), the total content of CLA and of polyunsaturated fatty acids content (PUFA) in milk. Flaxseed enhanced also the α -linolenic acid content and the n-3 fatty acid content of milk. Protection from solar radiation during summer did not affect yield and gross composition of ewe milk. Milk from ewes exposed to solar radiation showed reduced LCFA and PUFA content, and CLA content. The administration of flaxseed to lactating ewes during summer can be considered a strategy to improve the milk fatty acid profile which undergoes a marked worsening, and meet the consumers' demand for cheeses naturally-enriched in healthy component.

32W

Red deer (*Cervus elaphus*) damage investigation in Foreste Casentinesi, Monte Falterona and Campigna National Park

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The Foreste Casentinesi, Monte Falterona and Campigna National Park has an area of about 36,000 ha and is located along the Apennine ridge, between the regions of Emilia Romagna and Tuscany. The forest area is about 79%, while areas used for agriculture and grazing are about 7.5%. In the Park there are wild boar (*Sus scrofa*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*); the only predator is the wolf (*Canis lupus*). The most common deer is the red deer, an intermediate feeder being able to browse woody vegetation or graze grasslands. The aim of this study was to assess the damage from red deer, based on the damaged surface and on the compensation that the Park annually paid during years 2006-2008. We used a GIS system that allowed us to obtain data on damaged areas subdivided by crop types (pasture, meadow pasture, arable, orchards and chestnut groves); it has also been possible to locate the orchards and chestnut groves for which compensation was paid. The nocturnal observation, the height of browsing and removed foliage permitted to discriminate between damage from different species. Pasture, meadow pasture and arable were damaged by red deer only in the first two years of our investigations (2006: 3,400 ha; 2007: 26,590 ha) and only in the Tuscan side of the Park, while in the Emilian side damages were caused by all the other ungulate. Orchards and chestnuts groves, instead, have been damaged in three years always in the same areas of Tuscan side. Total annual compensations paid for pasture, meadow pasture, arable, orchards and chestnut groves were very low, respectively € 2,192 for 2006, € 694 for 2007 and € 350 for 2008. From this analysis, an enormous damage to the coppice together with the lack of compensation is also emerged; therefore the local Mountain Community requires a reduction of red deer population, creating a conflict with the purposes of the Park.

33W

Yeast supplementation on *in vitro* rumen fermentation: comparison of inactivated and live cells of *Saccharomyces cerevisiae*

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Aim of the trial was to compare the effects of inactivated (IY) and live (LY) cells of *S. cerevisiae* on *in vitro* fermentation of a total mixed ration (TMR) with 60:40 forage:concentrate ratio that was incubated into batch cultures of mixed rumen microorganisms. Rumen fluid was obtained from twelve-rumen fistulated sheep that had been assigned randomly to the following treatments: four for the control group, four receiving 3.5 g IY/day per head and four receiving 1.5 g LY/day per head, during at least 10 days. Rumen pH in the incubation medium after 24 h of incubation tended to be lower when LY was added, whereas IY had no effect. Both yeast supplements affected total volatile fatty acid (VFA) composition; LY showed greater effects than IY, with increased VFA production. The highest values of ammonia nitrogen (P=0.006), total gas production (P<0.001) and *in vitro* dry matter disappearance (IVD) (P<0.001) were observed with LY. Methane production increased with both yeasts, and the highest values were observed with LY (P<0.001). Differences were also observed in the fermentation kinetics of the diet, with greater cumulative gas production at 24 h and average fermentation rate with LY than with IY, although the asymptotic gas production was not affected. Results show that LY can affect rumen fermentation to a slightly greater extent than IY, although both products require a regular administration and adaptation of the rumen microbial population for the stimulatory effects to become apparent.

34W

Effect of dietary inclusion of *Spirulina platensis* on nutrient digestibility in the Guinea pig

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This study aimed to study the nutrient digestibility of diets with or without inclusion of *Spirulina platensis* (a blue-green microalgae containing 65.8% crude protein, with potential antiobesity, hypcholesterolemic and antioxidant actions) in the Guinea pig (*Cavia porcellus*) by comparing a control diet (diet C) without *Spirulina* and an experimental diet (diet S) with 3% of *Spirulina*. The two diets were isoproteic (16.7 vs 16.6%) and isoenergy (16.8 vs 16.9

MJ/kg, for C and S diet, respectively) and were supplemented with 150 mg vitamin C/kg diet. Twenty adult guinea pigs of both sexes were divided into 2 homogeneous groups, individually housed in digestibility cages, and fed the 2 experimental diets during an adaptation period of 7 days and then the subsequent digestibility trial lasting 5 days. During the digestibility trial, feed consumption was measured and the faeces were collected individually for chemical analyses. ANOVA tested the effect of the diets, of the sex, and their interaction. Sex did not influence nutrient digestibility. The nutrient and energy digestibility of the 2 experimental diets appeared similar, contrary to the reduction in digestibility of nearly all nutrients observed in rabbits when fed Spirulina supplement. Spirulina inclusion increased fibre fraction digestibility, particularly haemicelluloses digestibility (49.6 vs 42.6%; $P < 0.05$). Potassium digestibility was significantly higher in S diet compared to C diet (88.7 vs 81.0%; $P < 0.001$), attributable to the high potassium content of Spirulina. Feed intake during the digestibility trial was slightly higher in the S group (0.23 vs 0.20 g DM/kg LW). The dietary inclusion of Spirulina did not affect nutrient digestibility substantially, and its supplementation in Guinea pig diets seems profitable for the coverage of protein requirements and animal health in general.

Acknowledgement

Research supported by Valman s.r.l., Isola Vicentina (VI)

35W

Effect of nucleotides on some biological markers of piglets performance

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Nucleotides are precursors of nucleic acids synthesis. They have important effects on the growth and development of rapid turnover cells. During the weaning a malnourishment condition affects the intestinal integrity and dietary nucleotides may become essential. Greater serum Cu and lower serum Zn are associated with risk of poor growth. We have investigated relationship between nucleotides' impact on piglets growth and serum Cu and Zn. Ten litters [HBI Fomeva11 x (LW x L)] were randomly assigned to two groups and during lactation (from 9 to 21 d of age) and post-weaning (from 22 to 35 days of age) they had ad libitum access to a basic feed, not containing AGPs, supplemented with 0 (control group, C; 53 piglets) or 1 g/kg of yeast extract nucleotides (treated group, T; 55 piglets). Zn and Cu content of the basic feed were 111 and 15 ppm, respectively. Piglets were weighed at the start of creep feed administration (9 d of age), at weaning (21 d), at 28 and at 35 d of age. At the same times on 10 piglets /group (2 piglets/litter), identified with an ear tag, serum Cu and Zn by a Jobin Yvon Ultima 2 (ICP-AES) have been determined. The data were submitted to GLM procedure [treatment (2 levels), sex (2 levels) and litter as fixed factors]. Nucleotide supplementation improved BW of piglets at 28 and 35 d compared with C group ($P < 0.05$), despite ADFI not differing between groups ($P > 0.05$). In T group serum Zn was lower at 28 and 35 d ($P < 0.05$) than in C group as serum Cu was not significantly different ($P > 0.05$). Nucleotides' beneficial impact on piglets growth may be due to an improvement of intestinal epithelium cells maturation. The lower serum Zn values observed in T group may be considered a response to a higher activity of the DNA and RNA enzymes Zn-dependent regulating replication and transcription cells.

36W

Shelf-life determination of organic Chianina meat vacuum-packed

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Maintaining quality and appearance is essential during the distribution and merchandising of meat, for this reason packaging has become an indispensable method to control organoleptic and chemical changes and to increase the shelf-life of this product. The aim of the trial was to test the shelf-life of meat derived from Chianina cattle reared under organic system and stored under vacuum packaging (VP). This trial was carried out on 8 Chianina females, slaughtered at 22 months of age; after one week of ageing, from each right half carcass 4 *longissimus dorsi* samples were taken: 1 sample was overwrapped with a traditional packaging (fresh meat) and 3 were stored under VP for 7, 14 and 21 days. To evaluate the effect of VP on qualitative parameters of beef, the following analyses were performed: pH, drip loss (%), cooking loss (%), tenderness (shear force kg), colour (L*a*b*C H), oxymyoglobin (%) and lipid oxidation (mg MDA/kg meat). Analyses were carried out on 32 samples: 8 before storage (fresh meat), 8 after 7 days of storage in VP, 8 after 14 days of storage in VP and 8 after 21 days of storage in VP. All data were analyzed by least squares means considering as categorical variable the storage time. Meat pH and water holding capacity were not affected by VP and values tended to be stable until 21 days. Meat tenderness increased significantly during storage time ($P<0.05$), as a consequence of carrying-on of the meat ageing in VP. As regard meat colour, L* value was not significantly influenced by VP, whereas a*, b*, C and H values showed discoloration in meat under VP ($P<0.01$), probably because the myoglobin changed in oxidized form, metmyoglobin, as confirmed by the significantly decrease of oxymyoglobin ($P<0.01$). Moreover the storage time didn't show significant effect on lipid oxidation of meat under VP. Therefore, the meat stored under VP showed some modifications in tenderness and colour in comparison with fresh meat, but from 7 to 21 days all parameters appeared to be stable over time, showing the good shelf-life of the Chianina meat obtained with organic system.

37W

Growth performance and meat quality of buffalo young bulls: 1. Effect of group size

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Aim of this study was to evaluate growth performance and meat quality of buffalo bulls maintained in different group sizes. Twenty-one young buffalo bulls were assigned into two different sized groups of 7 (group LS) or 14 (group HS) individuals. The groups were homogenous for body weight (337 ± 67 kg) and age (455 ± 72 d). Total pen area (3.2 indoor + 3.2 outdoor m^2 /animal) was kept constant in the two groups. All animals were fed the same diet [CP 14.7% dry matter (DM); Milk FU 0.91/kg DM]. Average daily gain (ADG) was calculated every 2 weeks, along with DM intake (DMI), calculated on pen basis. On a subgroup of 4 individuals per groups, carcass traits were recorded at slaughter. Meat quality (hardness, cohesion, chewiness, WB shear force) and meat colour (L*, a*, b*) were evaluated on 7-day aged muscles (*Longissimus dorsi*, *Semitendinosus*, *Semimembranosus*). DMI and carcass traits were analyzed by one-way ANOVA; ADG, calculated only for 7 animals from group HS, and meat quality parameters were analyzed by a linear mixed model for repeated measures. There was no significant effect of group size on DMI (8.65 vs 8.51 kg DM/day for LS and HS animals, respectively; SE 0.41), ADG (0.89 vs 0.96 kg/d; SE 0.048), carcass weight (249 vs 260 kg; SE 8.13) and dressing percentages (50.6 vs 50.8% ; SE 1.12). All carcasses from the two groups were classified O, for carcass conformation, and 4, for fat score, according to SEUROP grading system. There were no differences between the groups for the any meat quality traits, although hardness (3.92 vs 4.80 ; SE 0.48; $P<0.1$) and chewiness (2845 vs 3659 ; SE 4.77; $P<0.1$) tended to be higher in larger group. In conclusion, group size did not affect growth performance, although meat quality tended to be lower in the larger group.

38W

Growth performance and meat quality of buffalo young bulls: 2. Effect of dietary level of maize silage

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Aim of this study was to evaluate growth performance and meat quality of buffalo bulls fed two diets with different maize silage contents. Compositions (kg/head/d) of low (LS) and high silage diets (HS) were the following: maize silage 3.0 vs 10.5 ; alfalfa hay 0 vs 2.0 ; barley 0.25 vs 0.75 ; wheat flour middlings 1.0 vs 1.0 ; wheat straw 3.0 vs 0 ; commercial concentrate for lactating buffalo cows 0 vs 2.5 ; commercial concentrate for young buffalo bulls 4.5 vs 0 . The diets were approximately isoenergetic [0.92 vs 0.91 Milk FU/kg dry matter (DM)] and isonitrogenous (14.7 vs 14.7 CP% DM). Fourteen young buffalo bulls (316 ± 70 days of age and 436 ± 67 kg

live weight) were assigned into the two dietary groups. Average daily gain (ADG) was calculated every 2 weeks, along with DM intake (DMI), calculated on pen basis. On a subgroup of 4 individuals per groups, carcass traits were recorded at slaughter. Meat quality (hardness, cohesion, chewiness, WB shear force) and meat colour (L^* , a^* , b^*) were evaluated on 7-day aged muscles (*Longissimus dorsi*, Semitendinosus, Semimembranosus). DMI and carcass traits were analyzed by one-way ANOVA. ADG and meat quality parameters were analyzed by a linear mixed model for repeated measures. No differences were observed for diet DMI (8.41 vs 8.64 kg DM/day for LS and HS diets, respectively; SE 0.44), as a consequence ADG did not differ between the groups (0.92 vs 0.89 kg/day; SE 0.04). All carcasses had the same carcass conformation (O) and fatness (4) scores. No differences were observed for carcass traits (carcass weight 250 vs 249 kg; SE 8.13; dressing percentages 52.1 vs 50.6%; SE 1.1) and any meat quality parameters. Therefore, maize silage can be either used as the primary source of roughage or in combination with other forages without affecting growth performance and meat quality.

39W

Microarray analysis in skeletal muscle of Chianina and Maremmana breeds

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Identification of genes controlling meat tenderness and correlated metabolic pathways can be easily and straightforwardly addressed by exploiting the large scale capacity of microarrays. We collected skeletal muscle samples from *Longissimus dorsi* (LD) of 7 Maremmana (M) and 10 Chianina (C) young bulls. Samples were classified into four categories (very hard, hard, tender and very tender) on the basis of phenotypic analysis, like Warner-Bratzler shear force, the index of myofibrillar degradation, the proteolytic activity and the insolubility of collagen. To assess the differences in skeletal muscle transcriptome, we used a custom microarray generated from *Bos taurus* ESTs. Reproducible patterns of differentially expressed genes were achieved between breeds and within the same breed analysing the 2 extreme degrees of tenderness (very hard and very tender). We performed 4 types of comparisons analyzing 2 samples for each breed: 1) very hard muscle, C vs M; 2) very tender muscle, C vs M; 3) the very tender muscle vs the very hard one within C; 4) the very tender muscle vs the very hard one within M. To establish the significance of the observed regu-

lation for each gene, we used the t-test and corrected the p-value for multiple comparisons with the Benjamini and Hochberg FDR. The statistical significance of the enrichment for the KEGG pathways of interest was computed using the hypergeometric test. With a $|FC| > 2$ and a p-value ≤ 0.05 , we identified 73 differentially expressed genes comparing very hard muscle between breeds, 53 of which are overexpressed in M, 20 in C. The comparison within Chianina samples showed 34 differentially expressed genes 18 of which are overexpressed in very tender C, 16 in very hard C. We are still confirming assays 2 and 4. Finally, a KEGG analysis of the differentially expressed genes in each comparison evidenced several metabolic pathways like energy metabolism.

40W

NIRS analysis of different meat sample preparations to discriminate feeding plan and genetic type effects in veal calves

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The aim of this study was to investigate the feasibility of using NIRS methodology to discriminate feeding plan and genetic type effects of three veal calves sample preparations. A portable NIRS instrument examined 128 *longissimus thoracis* (LT) of raw (RA), ethanol-prepared (ET) and freeze-dried (FD) samples from 32 veal calves. The calves, 16 Friesian (F) and 16 Crossbred (C) were fed on milk replacer and maize silage; in addition they received 65 kg/calf (8LF+8LC) or 100 kg/calf (8HF+8HC) of maize grain. LT samples were analysed for: water, protein, fat and haem iron content, drip and cooking losses, colour (L^* , a^* , b^* , Chroma and Hue), Warner-Bratzler shear and fatty acids composition of intramuscular fat. Chemometrics was performed by Partial Least Squares (PLS) method to obtain the whole 4x4 cross-validated matrix of the distances and the solutions for the average main effects. The average cluster of the four groups means was built by the validation matrix. The same PLS method was applied to a set of 12 laboratory chemical and physical analyses (Lab) and to fatty acids (FA) composition of intramuscular fat. The matrices reached different R^2 levels: 0.75 (RA); 0.84 (ET); 0.46 (FD); 0.46 (Lab); 0.13 (FA). Cluster from NIRS of raw samples corresponded to the same pattern obtained by the Lab and FA, which approached the HF to LC and the LF to HC groups. Regarding the NIRS of ET, both effects appeared very high ($R^2=0.83$) while for FD the feeding plan differences appeared only at medium level ($R^2=0.39$). It was concluded that NIRS scan of RA muscle produce final results achieved after a wide set of laboratory analyses of meat. NIRS analysis of ET samples showed other intrinsic differences which should be further investigated.

41W

Carcass and meat quality of growing-finishing pigs reared on sowed crops

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Pasture-based swine management can offer the producer low production costs, and a sustainable method for producing meat. Aim of this trial was to study the carcass and meat quality of 16 commercial hybrid pigs (8 castrated males and 8 females) reared outdoor from April to October (170 days) in Piedmont. The initial and final live weight (LW) were 90 and 141 kg; animals were allowed to forage pea, clover, beet and alfalfa pastures in a crop-pasture rotation on different paddocks. A concentrate was fed to supply 50% of estimated energy and protein requirements. Data collected at slaughtering were: hot carcass weight and yield, lean cuts (neck, ham, shoulder and loin) and fat cuts (backfat, belly, collar fat, flare fat) weight, backfat thickness, pH45 and pH24. Samples of *Longissimus dorsi* from each carcass were analysed for: water, fat, protein, colour (L*, a*, b*, Chroma, Hue), drip and cooking losses, shear force; fatty acid composition of intramuscular fat. Differences between males and females were carried out by ANOVA of SPSS. During the trial, the stocking rate (SR) ranged from 109 to 2347 kg/ha LW (average SR: 662 kg/ha LW); forage dry matter intake (DMI) increased from 0.32 to 2.85 kg/d, depending on the period and pasture; average daily gain (ADG) was 0.29 kg. At slaughtering, no statistical difference were observed between males and females. However, males tended to have a higher carcass yield (76.1% vs 74.0%), backfat thickness (23.0 mm vs 19.6 mm), fat cuts (25.6% vs 23.9%), meat fat (1.5% vs 1.0%) and SFAs (38.9% vs 38.4%) than females. Meat of females showed better water holding capacity but less tenderness. Results showed that pasture-based swine management should be especially appealing to limited-resource farmers due to low inputs needed; pasture can be used to replace 50% of the needs, helping to save on grain and protein costs, without affecting carcass yield and meat quality.

42W

Validation of standards for beef sensory evaluation

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As beef sensory evaluation has intrinsic pitfalls (cooking, vari-

ability between muscles and individual animals), a descriptive training program was designed to standardize evaluation practices among evaluators. Standards related to three levels of intensity (high, medium, low) for the most common meat sensory attributes (tenderness, chewiness, juiciness, flavour, odour) were developed by an experienced panel of 10 assessors (Panel A). Different cooking methods (grilling, microwave and boiling) were employed in order to define different odour/flavour intensities. Tenderness thresholds were identified using different muscles (*Psoas major*, *Longissimus dorsi*, *Extensor carpi radialis*). Juiciness levels were obtained by cooking to different internal temperatures (65, 70, 75°C). Standards defined by Panel A were used to train a new panel without previous experience (Panel B). After training Panel B re-assessed the three levels of intensity of each attribute in blind conditions. Data were analysed using ANOVA with panel, intensity and their interaction as factors. Panels rated attribute intensities similarly, apart from chewiness where a significant panel x intensity interaction ($P < 0.05$) was observed. This was possibly due to different chewing behaviour of panellists. These results provided external validation of the standards to be used for beef sensory evaluation. Subsequently, both Panels A and B performed a quantitative descriptive analysis to evaluate the same beef samples originating from Podolian young bulls finished in either intensive or extensive conditions. Data were subjected to ANOVA with panel, finishing system and their interaction as factors. No significant interactions panel x finishing system were observed. This indicated a high reliability of the panels in terms of consistency of results (i.e. different panels yielded similar results). In conclusion, our standards for beef sensory evaluation simplified training and produced high panel reliability.

43W

Lypolytic pattern of meat from autochthonous lamb reared in continental Southern Italy

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The effect of genotype and muscle on lipolytic pattern of meat were evaluated in 50 lambs of Altamurana, Bagnolese, Laticauda, Leccese, and Gentile di Puglia breeds. Lambs investigated were all males and slaughtered at 50 days of age. Fatty acid profile was measured on two different muscles (*Longissimus dorsi*, LD, and *Semimembranosus*, SM). Lipids were extracted according to the method described by Bligh and Dyer (1959) and then methylated according to ISO-IDF (2002). Gas-chromatographic analysis was performed with a HP88 capillary column (length 100 m, internal diameter 0.25 μm , film thickness 0.25 μm); fatty acids were expressed as percent of total methylated fatty acids. Muscle effect was not significant, while genotype effect revealed significant differences in meat fatty acid profile. Meat from Laticauda and Gentile di Puglia showed higher content in saturated fatty acids (SFA) and Laticauda, Gentile di Puglia, and Leccese had higher levels of monounsaturated fatty acids (MUFA) than the other breeds. Apart from breed SFA and PUFA were lower and higher, respectively, than previous findings on lambs slaughtered at the same age. Content of $\omega 3$ and $\omega 6$ fatty acids was the highest in meat from Altamurana and Gentile di Puglia lambs, respectively. PUFA/SFA was higher than 0.3 in all breeds, indicating a good nutritional value of the meat. CLA content also evidenced significant differences among breeds. Atherogenic Index and Thrombogenic Index found in this study were lower than those reported in other studies involving Merino lamb. Overall, the study of lipolytic pattern of meat from autochthonous lamb reared in continental Southern Italy evidenced that lamb meat exhibits appreciable nutritional value.

44W

Meat products as a source of dietary unsaturated fatty acids

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This work aimed to study the effects that meat from different species as donkey, buffalo and podolian young bulls has on fatty acids composition of salami and bresaola and to compare them with traditional salami and bresaola. Fatty acids (FA) composition of total lipid extract of each sample was determined by gas chromatography analysis at the end of ripening time. Each FA concentration was expressed as a percentage of total FAME and nutritional indices were calculated. Data were analyzed with ANOVA using animal type as the main effect. Different meat caused significant differences in the unsaturation level of fatty acids of salami and bresaola. Salami manufactured with meat from donkey showed

lower ($P<0.05$) saturated fatty acid (SFA) and higher ($P<0.01$) polyunsaturated fatty acids (PUFA) than salami from buffalo meat, Podolian beef and pork; the highest fatty acids $\omega 3$ content ($P<0.01$) were found in donkey and Podolian young bulls salami. In addition salami from donkey meat and podolian beef showed better nutritional indexes than other salami showing lower $\omega 6/\omega 3$ ratio ($P<0.05$) and lower atherogenic and thrombogenic indices ($P<0.01$). Analogously, donkey and Podolian beef bresaola showed lower SFA percentage ($P<0.01$) and higher PUFA content ($P<0.01$) with higher fatty acids $\omega 3$ ($P<0.01$) than beef and buffalo bresaola. In particular, salami and bresaola from donkey and podolian meat had a higher content of very long chain (VLC) fatty acids, such as eicosapentenoic ($P<0.01$), and docosahexenoic ($P<0.05$) acids than traditional meat products. These results demonstrate the possibility to produce fermented and cured meat products with a high value of unsaturated fatty acids using alternative meat instead of traditional pork or cattle.

45W

Effects of slaughter weight on carcass traits and meat quality of Casertana pigs

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The present research aims to extend our knowledge of the Casertana breed, and to evaluate the effects of slaughter weight (W) on carcass traits and meat quality (pH, colour and collagen) of this pig. Eighteen barrow Casertana pigs were housed in 4 single huts (4 pigs for each) and fed on the basis of live weight (from initial weight to 60 kg; from 60 to 100 kg; from 100 kg until slaughter). Diets were supplied based on 9% of metabolic weight. Animals were slaughtered at two live weights/ages: 8 barrows at 151.2 \pm 2.3 kg/364.7 \pm 1.7d (A); 8 barrows at 181.8 \pm 2.0 kg/445.7 \pm 1.7d (B). Hot carcass weight were recorded and dressing percentage was calculated. Backfat thickness was measured and carcasses were dissected into commercial cuts (lean cuts: ham, loin, neck, shoulder; fatty cuts: backfat, belly, jowl, kidney fat). *Longissimus lumborum* muscle was collected for intramuscular collagen (IMC) analyses, collagen and crosslink (hydroxylsilypyridinoline = HLP) concentration. Mean values of data have been compared using the Student's t-test. Compared to A pigs, B pigs had higher ($P<0.01$) carcass weight (149.3 \pm 2.1 vs 123.0 \pm 2.3 kg), dressing percentage (+0.8%), ham yield (+1.0%), backfat thickness (+34.1%) and fatty cuts yield (+2.5%), and lower loin yield (-1.2%; $P<0.01$). Weight did not affect lean cuts yield, pH and colour of the meat, IMC concentration, and IMC maturity crosslinking (moles of HLP/mole of collagen). Muscle HLP crosslink concentration was higher in B pigs than in A pigs (7.5 \pm 0.82 vs. 44 \pm 0.91 $\mu\text{g}/\text{mg}$, respectively; $P<0.05$). Results from

this study indicate that increasing slaughter weight resulted in a increase in both dressing percentage and carcass fatness. In addition, the heavier pigs have produced a meat that could be tougher than that of lighter pigs but that could have a positive effect on yield from a technological point of view.

46W

Slaughtering traits and meat quality of Modicana and Charolais x Modicana crossbreed calves slaughtered at the same finishing grade

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Aiming to compare some slaughtering and meat quality traits of animals belonging to a pure rustic breed and to its crossbreed, reared in the same breeding system and slaughtered at the same finishing grade, 20 entire male animals were studied: 10 Modicana pure breed (MO) and 10 Charolais x Modicana (CM) crossbreed. Calves were reared with their dams in extensive conditions (Ragusa, Italy) until weaning age (about 8-10 months); successively, until slaughtering which took place at 20 months, according to the local market preferences and the finishing grade, they fed concentrate and meadow hay offered ad libitum. At slaughtering, hot carcass weight and linear measurements were recorded to calculate Carcass compactness index and Lateral conformation and the carcasses were classified on the basis of conformation and fat cover using the SEUROP quality classification. One week after slaughtering, the Longissimus thoracis muscles (T7-T8), were removed and used to determine the meat quality traits. Data was performed using a one-way ANOVA. Carcass weight (MO 377kg vs CM 417kg; $P=0.044$) and SEUROP scores (MO 4.25 vs CM 5.25; $P=0.046$) showed significant differences; similar values were observed for fatness scores (MO 3 and CM 3.25), Carcass compactness index (MO 2.66 kg/cm and CM 2.92 kg/cm) and Lateral conformation (MO 30.41% and CM 30.51%). Meat quality showed no significant differences for pH (MO 5.70 vs CM 5.69), Luminosity (MO 43.41 and CM 42.03), hue angle (MO 21.71 and CM 23.76), cooking losses (MO 30.13% and CM 31.57%), tenderness (MO 4.40 kg/cm² and CM 4.29 kg/cm²), protein (MO 22.45% and CM 22.70%) and fat (MO 1.69% and CM 1.50%). The redness index was significantly higher in the Longissimus thoracis muscle of CM (MO 17.18 vs CM 20.41; $P=0.048$). The results of the present research should encourage Sicilian farmers to grow and finish the young male stock of these native cattle breeds in their farms of origin.

47W

Meat quality of Sardo-Bruna beef with diurnal grazing

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A study was carried out to evaluate meat quality of grazing Sardo-Bruna beef. After weaning, 5 bulls and 5 heifers (average 8.5 months of age) were grazed only during the daylight and supplemented with a mixture of concentrate and of meadow hay. Males and females were slaughtered at about 500 and 400 kg live weight, respectively. Age, live weight, carcass weight, conformation and fatness of carcass (SEUROP-system) were detected. On M. Longissimus dorsi were determined meat colour (CIELab-system) and pH at level of last rib and dry matter, crude protein and fat contents between 5th and 7th rib. Data were analysed by one way ANOVA procedure to evaluate differences between sexes. Age (17.0±1.4 vs 14.8±0.4 months), live weight (536±53 vs 412±19 kg), warm (289±30 vs 206±15 kg) and cold (283±29 vs 203±15 kg) carcass weight, cold dressing percentage (52.9%±1.4 vs 49.4%±1.9) were significantly ($P=0.01$) higher in bulls than heifers. As regard SEUROP carcass classification, carcasses of bulls were constant in conformation but variable in fatness (60% U2 and 40% U1), while carcasses of heifers were constant in fatness and variable in conformation (60% U2 and 40% R2). pH and colour did not differ between sexes (pH 5.50±0.08 and 5.56±0.17, L 39.7±2.0 and 39.4±2.7, a 18.7±2.5 and 20.0±1.2, b 6.2±3.7 and 8.6±3.0 in bulls and heifers, respectively). Dry matter and fat content of L. dorsi were higher in heifers than bulls (DM 26.0%±0.5 vs 23.9%±0.6, $P<0.001$; fat 2.7%±1.1 vs 1.4%±0.6, $P=0.04$), while crude protein content did not differ between males (21.5%±1.0) and females (21.0%±1.2). In conclusion, Sardo-Bruna beef, grazing during the daylight and supplemented with concentrate and hay, can produce meat not different between sexes and similar to that of specialized breeds, slaughtered at the same age.

48W

Performance of Sardo-Bruna beef with diurnal grazing

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A study was carried out to evaluate productive and economic performance for meat production of Sardo-Bruna beef. After weaning (8.5 months of age), 5 bulls and 5 heifers – respectively 287±26

and 248 ± 28 kg live weight – were grazed during the daylight and housed during the night. A mixture of barley-corn meal, a complete mixed feed and meadow hay were used as supplementation and administered, before and after grazing, according to grass quality. It was decided to slaughter males and females at a suitable weight for the breed, about 500 and 400 kg live weight respectively. Live weight at slaughtering, daily growth and economic parameters were detected. Data were analysed by one way ANOVA procedure to evaluate differences between sexes. At slaughtering body weight was in accordance with that fixed as target (males kg 536 ± 53 , females kg 412 ± 19). Average daily gain was slightly higher in bulls than heifers (g/d 1027 ± 202 vs 822 ± 63 ; $P=0.063$). Bulls, consequently, needed more time to reach the slaughter weight (days 244 ± 35 vs 199 ± 16 , $P=0.032$). The daily cost of supplementation as such, was greater in males than females (€/head/d 1.083 ± 0.103 vs 0.872 ± 0.029 ; $P=0.002$) as consequences of a higher intake of concentrate (kg/head bulls 3.9 ± 0.6 , heifers 2.9 ± 0.2); if it was considered per kilo of growth (€/kg bulls 1.106 ± 0.339 vs heifers 1.066 ± 0.089) no differences were detected. Assuming 2.5 €/kg live weight as price, the overall gain during fattening – defined as difference between increase value and cost of supplementation – was higher (not statistically different) and more variable in males than females (€/head 356 ± 126 vs 235 ± 28). In conclusion, food supplementation to grazing young bulls and heifers may be considered an efficient and economical rearing technique of Sardo-Bruna beef on dry farms of Sardinia.

49W

The changes in oxidative stability of Sarda lamb meat during frozen storage

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Lipid oxidation is the primary cause of loss of meat quality during frozen storage. Raw samples of tight muscles (*semitendinosus*, *semimembranosus* and *femoral biceps*) from Sarda suckling lambs were frozen at -20°C for up to 6 months and the oxidative changes in their lipids were evaluated by measuring the values of malondialdehyde (MDA) by TBARS test. Samples come from 48 suckling lambs divided into 2 groups with different feeding systems: 24 were raised indoor (group IN) and 24 followed their mother outdoor on pasture (group OUT). Lambs were slaughtered at 28 days of age. After 24h of refrigeration at 4°C , the tight muscles were divided into different pieces (about 250 g each) and stored in a household freezer at -20°C for 30, 90, 150 and 180 days. MDA levels were measured with spectrophotometer and were expressed as mg MDA/kg meat. A frozen storage temperature of -20°C was adopted because it is commonly used for domestic purposes. Data were analyzed using a model including storage, feeding system and their interaction. The MDA levels were significantly influenced by feeding system, storage and their interaction. The increase in the values of MDA was not statistically significant in the IN group samples. The values of MDA increased significantly in the OUT group at 180 days of storage likely due to a higher content of polyunsaturated fatty acids. The results showed that the storage of meat at -20°C for extended periods does not prevent the oxidation process but certainly slows it down. The values of MDA detected in both experimental groups are within the limits of rancidity of about 1-2 mg MDA/100 g of product indicated by Watts (1962).

Acknowledgement

The research was funded by the University of Sassari (F.A.R. grant)

50W

Cluster analysis for the study of the relationship among some slaughter and meat quality parameters and different genotypes of rabbit: preliminary results

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The cluster analysis (CA) was applied to slaughtering data, chilled carcass composition, reference carcass characteristics and meat colour parameters of two different genotypes rabbits (25 Italian local population "LP" and 32 hybrids "HP") reared in colony cages under conventional system. The aim of the trial was to identify quality parameters that can discriminate rabbit meat from different genotypes. The CA was applied with the "Sum of square" methods on different groups of parameters considered separately or in combination. The groups were identified by the analysis and their percentage distribution within the two genotypes was calculated. Results showed that meat colour parameters and slaughtering data mainly allow to differentiate the two genotypes. In fact CA, applied to these parameters, identified three groups of rabbits: Group 1 (12 rabbits) was constituted by only LP (48% of total), Group 3 (14 rabbits) by only HP (44% of total), Group 2 (31 rabbits) by both genotypes (42% and 58% respectively). The three groups showed different characteristics: Group 1 had lower percentages of full gastrointestinal tract in comparison to Group 3 (17.23 *vs* 23.10; $P < 0.01$) and higher values of dressing out (61.38% *vs* 56.35%; $P < 0.01$), kidney (1.04% *vs* 0.99%; $P < 0.05$), redness (3.75 *vs* 3.02; $P < 0.05$) and Chroma (6.02 *vs* 4.59; $P < 0.01$). Group 2 showed intermediate values for many parameters. The data obtained in this trial showed that cluster analysis could be considered useful to discriminate genotypes using only some slaughter and meat quality traits.

51W

Effect of different cage size on performance of rabbit does

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Performance, paw injuries and mortality of rabbit does reared in cages of two different types were evaluated. Two hundred fifty-two

does were randomly divided into two groups and individually housed in standard wired cages (SC) sized 83×38×32 cm (0.10 m³) or in bigger cages (BC) sized 113×46×46 cm (0.24 m³), nest included. Each BC was equipped with a foot plastic mat covering about 25% of the cage floor. All performance were evaluated during 4 consecutive reproductive cycles. Parametric and non-parametric variables were statistically analyzed with ANOVA and Kruskal-Wallis test, respectively. Morbidity and mortality data were compared by using the Yates χ^2 test. Significance was declared at $P < 0.05$. Does body weight was higher in the BC group (4322 *vs* 4241 g). However, the same group showed lower feed efficiency index (kg of pups weaned/kg of feed ingested). Numbers of alive-born per kindling (BC, 7.3 *vs* SC, 7.5), number of weaned pups (BC, 6.2 *vs* SC, 6.0), and milk yield (BC, 115 g *vs* SC, 109 g) were not influenced by cage size. Body weight of weaned pups was not different between cages as well (BC, 599 g *vs* SC, 595 g). Animals housed in SC had 3.5-fold higher risk of mortality (15.7% *vs* 3.7%). Frequency and seriousness of paw injuries increased during the trial. During the 4th and last cycle the percentage of animals affected by this pathology were equal to 66% and 46% in the SC and BC groups, respectively. Based on the obtained results, it can be concluded that increased cage size and use of foot mat significantly reduce mortality and paw injuries, and increase does body weight; however, they worsen the feed efficiency index. This last effect could be attributed to a higher locomotory activity, but such hypothesis needs to be experimentally confirmed with further behavioural studies.

52W

Use of two different formulations of monensin sodium in broiler chicken diet: effects on coccidiosis control and production

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A 5-week trial was conducted in order to evaluate two different formulations of monensin sodium (powder and bi-modally protected granule – European Patent number 0197188) in terms of prophylactic efficiency and production performance of broiler chickens. Three-hundred and fifty 5-day-old Cobb 700 chicks were randomly assigned to 3 groups and infected with 8×10⁴ Eimeria spp. sporulated oocysts. All groups were fed ad libitum. Diets were supplemented with 100 ppm powder monensin sodium (group P, n=150) or 100 ppm bi-modally protected monensin sodium (group BMP, n=150). The remaining chicks were used as control (C, n=50).

Feed supplied and broilers' weight were recorded weekly. A total of 96 animals were randomly selected at slaughtering to quantify the number of oocysts per gram of intestinal content. Parametric and non-parametric variables were statistically analyzed with ANOVA and Mann-Whitney U or χ^2 tests, respectively. Significance was declared at $P < 0.05$. Feed supplied (overall mean: 3.2 kg head⁻¹ trial⁻¹) was similar among groups. Mean weight gain (overall mean: 47.8 g) was not influenced by diets. Necropsies showed significant differences concerning both the percentages of infected animals by *Eimeria* spp (BMP: 71%, P: 83%, and C: 100%) and the number of oocysts per gram of intestinal contents (BMP: 476.21, P: 1,574.57 and C: 10,428.50); all groups were significantly different from each other (Tukey-Kramer test). The results of this experiment suggest that the two monensin formulations could be effective treatments in control of chickens coccidiosis.

53W

Raising lactating rabbit does by PROBIOTAN at 1% integration or by medical prevention

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The aim of this study was to investigate the feasibility of using PROBIOTAN (oily extracts of stabilised vegetables by *Glycine S.*, *Menta P.*, *Malus P.*, *Centaureum U.*) as alternative of medical prevention in the lactating rabbit does cycle. Two groups of rabbit does, derived from an Italian ANCI selection scheme of *Macchiata italiana* and *Bianca Italiana* strains, were subjected to a mild rhythm and artificially inseminated 31 d pp with weaning at 40 d. Productive performances of the does (number of alive and dead kits at birth), litter size and litter weight at 3 d, and at 19 d were recorded. The does, equally allocated to the control (M) medicated by three-antibiotic prevention active and to experimental groups (P) integrated by PROBIOTAN at 1%, were weighed at 3 d postpartum (35 d since conception) and reweighed at 19 days. The total intake of pelleted feed consumed *ad libitum* (crude protein 16.5%, crude fibre 15.7%) from kindling until 19 following days, was also controlled. The experimental P diet, was administered from 3d to 19d pp. A total of 44 kindling involved 307 living kits. A significant and relevant reduction of 0-19d mortality (11.1% for P vs. 19.8% for M: Prob < 0.037 by Fisher exact test) was emerged. Performances of does, analysed by linear models, were unaffected by M or P regimen as regards to liveweight at 3d (avg. 4349±562 g) and at 19d (4330±480 g), to feed intake (5588±990 g), to litter weight at 3d (607±135 g) and at 19d (1820±581 g). It was concluded that this oily extract showed interesting result - because the control was medicated already - which should be confirmed in longer trials.

54W

Drivers of overall liking for organic and conventional rabbit

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Organic rules markedly affect farming practices, which in turn may influence meat quality and acceptability. The latter is a function of several attributes and their interactions. This study was undertaken to compare organic and conventional rabbit acceptability and investigate the main attributes moving their liking. Fifty consumers with an average age of 44 years and with a balanced distribution with respect to gender participated in the test. Each participant evaluated the two products in a randomised order. For each product, consumers expressed an overall liking and a liking according to the following sensory inputs: taste/flavour, juiciness, residual and tenderness. Likings were rated on a 9-point hedonic scale labelled at the left end with "extremely unpleasant" (1), at the right end with "extremely pleasant" (9) and at the central point with "neither pleasant nor unpleasant" (5). The Student's T-test was used to compare products. The relationship between overall liking and attribute liking was analyzed by linear regression analysis followed by slope analysis. Consumers rated both products at scores well above the central point (range 6.35-7.72). No attributes showed significant differences between the two products ($P > 0.05$). The slope analysis showed that taste/flavour ($k=0.54$) and residual ($k=0.42$) may be more important than tenderness ($k=0.39$) and juiciness ($k=0.33$) in orienting consumer preference for rabbit meat. These results indicate that meat from both organically and conventionally reared rabbits was characterised by a good eating quality, while organic roles had no detrimental effects on product liking, thus suggesting the adoption of marketing strategies based on information about process characteristics and signalling of ethical attributes.

55W

Effect of lycopene addition on the survivability of liquid-stored rabbit spermatozoa

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Lycopene, as an antioxidant, may help to maintain semen quality, however its effect on rabbit spermatozoa has never been studied. The aim of this study was to determine the effects of the addition

of increasing amount of lycopene on *in vitro* rabbit semen quality after 48 h of liquid storage. Six pools of rabbit semen were each divided into 3 aliquots that were extended with a Tris-based extender containing 0 (control), 0.05, or 0.1 mg/mL lycopene, respectively. Sperm mass and forward motility (light microscopy), viability (SyBr-PI staining), DNA integrity (Acridine orange staining) and osmotic-resistance (HOS-test) were determined on both fresh semen and stored one after 48 h at 5°C with and without a lycopene supplement. After 48 h of liquid-storage a significant reduction ($P<0.05$) of sperm motility, viability, osmotic-resistance and acrosome intactness was observed in rabbit spermatozoa with respect to the initial quality of fresh semen in all treatments, whereas DNA integrity in lycopene added samples did not differ from that of fresh semen. At 48 h of storage the semen added with both 0.05 and 0.1 mg/mL lycopene maintained a significantly higher forward motility, viability and acrosome intactness compared to those of semen stored without lycopene ($P<0.05$). Moreover, with the higher dose of lycopene, also total mass motility and DNA integrity were better compared to those scored in semen stored without lycopene ($P<0.05$). In conclusion, lycopene is able to reduce the natural damage that occurs in rabbit sperm cells during 48 h of liquid-storage and it acts in a dose-dependent way.

56W

Structural and functional damages in rabbit spermatozoa following vitrification

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Despite increasing attention being paid to the vitrification as reproductive biotechnology for gametes preservation, its consequences on rabbit sperm quality have not been adequately investigated. This work aimed to evaluate the effects of vitrification either with presence or absence of cryoprotectants (CPAs) on motility and structural integrity of rabbit spermatozoa. Six pools of rabbit semen five-fold diluted with a Tris-based extender were each divided into 3 equal parts, cooled and respectively vitrified 1) without CPAs; 2) with 10% DMSO and 6% trehalose; 3) with 10% DMA and 6% trehalose. Vitrification was made by dropping 80 μ L of semen directly into liquid nitrogen to form frozen spheres. Sperm motility (light microscopy), viability (SyBr-PI staining), DNA integrity (Acridine orange staining), osmotic-resistance (HOS-test) and ultrastructure (Scanning Electron Microscopy; SEM) were assessed on both fresh and vitrified samples after thawing at 60°C for few seconds. Motility was completely absent in sperm after vitrification in all treatments, whereas DNA integrity resulted lower only in samples vitrified with DMSO/trehalose ($P<0.05$). Sperm viability, osmotic-resistance and acrosome integrity were also affected by vitrification ($P<0.05$), however lower recovery rates of viability and acrosome intact spermatozoa were found post-thaw when DMSO/trehalose was used compared to the other treatments ($P<0.05$). SEM observation revealed that most of the vitrified spermatozoa had acrosome lost, broken plasmamembrane, detached head and acute tail flexion, however the spermatozoa vitrified with DMSO/trehalose were most damaged than those vitrified with DMA/trehalose or without CPAs. The results show that *in vitro* postwarming quality of vitrified rabbit spermatozoa is greatly impaired both with presence or absence of CPAs, however both the DMA/trehalose and CPAs-free vitrification seem better protect the sperm cells against cryoinjuries than DMSO/trehalose.

57T

Identification of polymorphisms in the sheep and goat *MITF* gene

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Microphthalmia-associated transcription factor (*MITF*) is a member of the Myc superfamily of basic helix-loop-helix zipper transcription factors that functions as a master regulator of melanocyte development. Mutations in the *MITF* gene having a wide range of effects on coat colour, from moderate spotting to complete absence of pigmentation, have been reported in mouse, rat, Syrian hamster, dogs, and Japanese quail. In humans, *MITF* gene mutations are responsible for Waardenburg syndrome type 2A. We recently analysed the cattle *MITF* gene and showed association between a few haplotypes and the spotted phenotype in Holstein and Simmental compared to non-spotted breeds. As a first step to evaluate a possible effect on coat colour pattern in sheep and goat, we sequenced portions of the *MITF* gene in these two ruminant species. Primers were designed on the cattle *MITF* gene sequence and amplified fragments were sequenced in a panel of 70 sheep (from 6 breeds) and 40 goats (from 4 breeds) with different coat colour patterns. Several polymorphisms were identified in both species. Haplotypes were reconstructed and evolutionary relationships were analysed constructing Median-joining networks. The identified polymorphisms will be useful to evaluate association with different coat colour patterns across breeds within species.

58T

The dilute coat colour locus is associated with polymorphisms of the melanophilin (*MLPH*) gene in the domestic rabbit

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Classical genetic studies in the domestic rabbit have shown that a recessive coat colour mutation (known as dilute) dilutes the black

to blue (grey) and the yellow to beige. To confirm the mode of inheritance and to obtain genetic materials for association studies we constructed a rabbit reference population crossing a blue buck with putative heterozygous black does. A perfect 1:1 mendelian ratio of black and blue rabbits was observed in the F1 population. Moreover, we selected three candidate genes (melanophilin, *MLPH*; myosin VA (heavy chain 12, myosin), *MYO5A*; and pre-melanosome protein, *PMEL*) whose mutations in other species have shown to cause similar phenotypes, and sequenced several gene fragments in a panel composed by rabbits from the reference population with black and blue coat colour and from different breeds. Several polymorphisms were identified in all investigated genes but only those discovered in the *MLPH* gene co-segregated with the normal (black) or dilute alleles. In addition, a deletion of ~50 bp detected in the dilute allele was genotyped in the rabbit reference population and in rabbits of different breeds, confirming that the mutated allele was present in homozygous condition only in blue rabbits.

59T

Analysis of copy number variations in the European rabbit (*Oryctolagus cuniculus*) genome

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With the advent of genome wide analysis methods to detect DNA content, such as array comparative genome hybridization (aCGH), it has been soon realized that copy number variations (CNVs) (DNA segments ranging from 1 kb to few Mb that present a variable copy number in comparison to a reference genome) represent a significant source of genetic variability in all vertebrates as well as in inferior species. The European rabbit (*Oryctolagus cuniculus*) genome has been recently sequenced at ~7X coverage and assembled (OryCun2 genome version) by the Broad Institute in the context of the Mammalian Genome Projects in order to improve the annotation of the human genome. To obtain a first CNV map of the European rabbit genome, we used a customized tiling array including ~720,000 oligonucleotide probes designed on the OryCun2 genome assembly and analysed genomic DNA samples of 3 rabbits (one male and two females) of different breeds compared to the reference DNA of another rabbit (female). CNVs were reported using 10 different algorithms developed for data segmentation and averaging their results as implemented in the CGHweb server. Chromosome X signals and self-self hybridization results of the reference sample served as quality indicators. Using a conservative approach, high confidence CNVs were

detected on most chromosomes. These results contributed to develop an initial CNV map of the European rabbit genome that can be used as reference in comparative genome analysis for other leporid species.

60T

SNP identification and expression study on pig fat genes associated with fatness traits

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Fat deposition is a crucial aspect of pig meat quality: a suitable marbling and a right balance between lean meat and fat in carcass are essential to produce high quality dry cured hams. Differences in intramuscular fat content and backfat thickness among pig breeds are reported by several authors but the genes associated to fat traits are poorly known. The aim of this study is to investigate on the expression level of fifteen genes involved in fat metabolism or associated to obesity in humans *ACACA*, *ACLY*, *FASN*, *ENO3*, *MTTP*, *PLTP*, *CES3*, *PCSK1*, *ALK7*, *LMNA*, *APP*, *ADRB2*, *PDE4B*, *SORT1*, *INSIG2* in skeletal muscle and adipose tissue of Italian Large White (ILW) and Italian Duroc (ID) pigs and to identify SNPs in these genes. cDNA was obtained from Semimebranosus skeletal muscle and fat samples collected at slaughterhouse from sib tested ILW and ID pigs. Quantitative Real Time PCR was carried out using the standard curve methods and reporting the data normalised to housekeeping genes identified for the studied tissues. Preliminary results on *ACLY*, *ACACA* and *FASN* genes indicate a different level of transcription (comparison assessed using the paired Student's t test of these genes between ID and ILW breeds both in fat (12 ID and 26 ILW samples; $P=0.02$, $P=0.004$, $P=0.08$, respectively) and skeletal muscle (25 ID and 32 ILW samples; $P=0.02$, $P=0.19$, $P=0.01$, respectively). In pig adipose tissue the three lipogenic genes are more expressed in ILW than in ID pigs. Up to now the SNPs *FASN* g.265T>C (Munoz *et al.* 2003), *ACLY* c.2956T>C, and *ACLY* c.3923T>C (both found by us using an in silico approach) have been analyzed in different breeds. The expression study on the other genes and the SNPs search are in progress in cosmopolitan and Italian local pig breeds. The study of transcription profiles and allelic variants of these genes could provide suitable markers to define their involvement on meat and carcass fat deposition in pig breeds.

61T

Study of the lipid droplet coat proteins PLIN1 and PLIN2 and corresponding genes in skeletal muscle of Italian heavy pigs

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Perilipin 1 (PLIN1) and 2 (PLIN2) are cytosolic lipid droplets coat proteins involved in lipid storage and mobilization, with major role in adipocytes and in non-adipose cells (as myocytes), respectively. *PLIN1* and *PLIN2* are candidate genes for fat deposition and for carcass and meat quality in pig. This study aimed to: localize PLIN1 and 2 proteins in pig skeletal muscles divergent for intramuscular fat (IMF); analyze *PLIN1* and *PLIN2* gene expression in muscle of pigs divergent for fat-related traits; study *PLIN1* and *PLIN2* polymorphisms and association with fat traits. PLIN1 and 2 were immunostained in pig muscles cross-sections from two groups divergent for IMF (n=4 each group). In the same muscles, *PLIN1* and *PLIN2* mRNA was quantified by quantitative Real Time PCR and compared by *t*-test. *PLIN2* expression was also analyzed in pigs divergent for visible intermuscular fat (VIF) and backfat thickness (BFT). Coding and non-coding regions of *PLIN1* and *PLIN2* were screened for SNP in different heavy pig breeds. Identified SNP were tested for association with production traits by linear mixed model analysis. PLIN1 localized in intramuscular adipocytes whereas PLIN2 localized in myofibers with high lipid content. *PLIN2* expression was higher in pigs with high IMF ($P<0.05$) and tended to be higher in pigs with higher VIF ($P=0.07$). Three and six SNP were identified in *PLIN1* and *PLIN2*, respectively. A *PLIN2* 3'-UTR SNP was significantly associated ($P<0.01$) with average daily gain, feed conversion ratio, lean cuts and hams weight estimated breeding values in 290 Italian Duroc pigs. In conclusion, PLIN1 and PLIN2 localized in correspondence to extra and intra-myocellular lipids, respectively. The differential expression in IMF and VIF divergent pigs and association result confirmed the *PLIN2* candidacy for fat traits. This study suggests that *PLIN1* and *PLIN2* can be markers for carcass quality in pigs.

62T

Association of myostatin (MSTN) gene polymorphisms with morphological traits in the Italian Heavy Draft Horse breed

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Myostatin (*MSTN*) is a negative modulator of muscle mass. Previously, we identified single nucleotide polymorphisms (SNPs) in horse *MSTN* gene that could be associated with morphological traits. In particular, two SNPs of the promoter of *MSTN* gene were statistically more frequent in heavy than in light horse breeds. In addition, other studies indicated that a SNP in the first intron of the *MSTN* is associated with athletic abilities in Thoroughbred horses. Here, we evaluated the effect of SNPs in the *MSTN* gene on several morphological traits measured in Italian Heavy Draft Horse (IHDH) breed. This horse breed is currently selected mainly for meat production. The linear type evaluation data of registered animals in the studbook were used to calculate estimated breeding values (EBVs) for 11 traits related to general aspects, trunk and leg traits. Two hundred and six horses (53 mares and 153 stallions) were chosen according to the accuracy of EBVs and genealogical information. DNA was extracted from hair roots and genotyped by PCR-RFLPs for 3 SNPs, two in the promoter and one in the first intron of the *MSTN* gene. Minor allele frequencies of SNPs varied from 0.117 to 0.147. Significant associations of markers with head size and expression ($P < 0.05$), direction of upper line ($P < 0.01$), and rear diameter ($P < 0.05$) were identified. These morphological traits have an important economic value for carcass and meat yields, and longevity of mares.

63T

Haplotype variability and genetic heterogeneity at the porcine *FTO* gene in different pig breeds

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Polymorphisms in the fat mass and obesity associated gene (*FTO*) are associated with common obesity in human cohorts. We recently showed that a single nucleotide polymorphism (SNP) in intron 3 of the porcine *FTO* gene is associated with fat deposition and correlated traits in Italian Duroc and commercial pig populations. To identify additional variability in this gene, that could have functional effects, we sequenced 13 fragments of *FTO* gene regions (including exon and intronic regions) in 44 pigs belonging to 8 pig breeds (Italian Large White, Italian Duroc, Italian Landrace, Meishan, Hampshire, Belgian Landrace, Cinta Senese and Casertana) for a total of ~5 kb for each animal. A total of 26 SNPs and one indel were identified. These polymorphisms were used to infer haplotype structure and linkage disequilibrium blocks.

Median-joining tree and phylogenetic dendrograms were constructed and nucleotide diversity, haplotype diversity and Tajima's D were estimated. The results indicated a quite high level of genetic diversity in this gene but comparable to other genomic regions we already analysed in pigs. These results can be used to evaluate potential signature of selection differences across breeds that are different in terms of fat deposition.

64T

Effects of genotype at wattle locus on reproductive parameters of Nero di Parma sows

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Wattles in pigs are cutaneous appendices on the ventral surface of the neck, whose function is still unknown. They are inherited as a single autosomal dominant gene (Wa^W), as the recessive allele (Wa^+) encodes for the absence of wattles. It has been previously assessed that genotype at wattle locus has a significant effect on Gompertz equation parameters for growth and body measures in Nero di Parma pigs, being wattled pigs lighter and smaller than not wattled pigs. In other species, as goat, also a positive effect of the presence of wattles on the reproductive efficiency has been reported. The aim of the study was to verify the effect of the genotype at wattle locus on the reproductive efficiency in Nero di Parma sows. To this aim 866 parities were considered, from 396 Nero di Parma sows. Of these, 268 did not showed wattles (genotype Wa^+/Wa^+) while 128 had one (no.= 17) or two (no.= 111) wattles (genotype $Wa^W/-$). The parameters considered were the age at parities, the total number of piglets born, born alive, stillbirth and weaned, the duration of the period between two subsequent parities and the number of teats. Data were analyzed by the analysis of variance, according to a model with herd, year, parity, genotype at wattle locus, and interactions of boar * sow within herd and parity * genotype at wattle locus. The reproductive parameters of sows were characterized by low values for average number of total born (7.8) born alive (7.4) and weaned (5.7). The genotype at wattle locus did not significantly affected ($P > 0.05$) the reproductive parameters of sows at all parities. However it was noted that $Wa^W/-$ sows had fewer teats than those with Wa^+/Wa^+ genotype (12.8 vs 13.2, resp.; $P < 0.01$). In conclusion it is likely that at current levels of reproductive efficiency the selection for the presence of wattles is not still a risk for the reproductive efficiency of Nero di Parma sows.

65T

Influence of milk quality and production protocol on proteolysis and lipolysis in Caciocavallo cheese

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The aim of this study was to assess the effect of milk source and of production protocol on proteolytic and lipolytic pattern of Caciocavallo cheese during ripening. The study involved six dairy factories in the rural area of Monti Dauni Meridionali. They varied in their source of raw milk: factories A, C, and E processed milk collected from farms located in the neighbouring area, and factories B, D, and F processed their own milk. A total of 24 milk samples were analysed: milk samples were collected from each farm on the day of the cheesemaking (two cheesemaking trials were performed for each farm) and analysed in duplicate for pH, total protein, fat, lactose content, and somatic cell count (SCC). Mesophilic bacteria and total coliform were enumerated in the milk and cheese samples using standard procedures; Caciocavallo cheese was characterized for fat, FFA, moisture, NaCl; total nitrogen (TN), water soluble nitrogen (WSN), non casein nitrogen (NCN), and pH 4.6 soluble-N. Water-insoluble and -soluble fractions of the cheeses were analysed by urea-polyacrylamide gel electrophoresis (PAGE). Data were analysed by ANOVA using the GLM procedure of SAS. The gross composition of bulk milk ranged between 3.59 and 6.08% fat, 3.28 and 3.83% protein, 2.46 and 2.98% casein, 4.54 and 4.79% lactose. SCC was always lower than 3 log₁₀ cells/mL; mesophilic bacteria were higher in B and D milk than in the other milk samples. Fat, protein, and casein content ranged between 29.7-49.5, 27.2-32.2 and 42.9-48.1% on dry matter, respectively at 180 days of ripening. Moisture content of Caciocavallo decreased during ripening ranging between 30.8 and 38.8% at 6 months of ripening. A, C, D and F cheeses accumulated the highest levels of WSN within the first three months of ripening with the small- and medium- sized peptides and free amino acids being further catabolized in the subsequent aging. Caciocavallo cheese showed differences in the evolution of proteolysis during ripening and in the intensity of the lipolytic process at the end of ripening. This study evidenced that the use of raw milk, of natural whey cultures, of rennet paste is able to influence markedly the proteolytic and lipolytic pattern of the Monti Dauni Meridionali Caciocavallo cheese.

66T

The effect of somatic cell count on Pecorino cheese sensory properties

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The somatic cell count (SCC) of milk, used as indicators of animal mammary disease, may vary widely because of endogenous and exogenous factors. Some studies have shown that an increase in SCC leads to a modification in the chemical composition of ewes' milk. The aim of this study was to determine the effect of two different SCC values on the most important sensory characteristics of Pecorino cheese. After thermization, bulk milk samples from 44 Sarda ewes with low (<500,000 mL⁻¹) or high (>2,400,000 mL⁻¹) SCC were used. Sensory analysis was performed after 2 months of ripening by a trained panel according to ISO 13299:2003. Samples of cheese were also subjected to analysis by electronic nose/metal oxide sensors. A two-way analysis of variance was applied to each sensory attribute with SCC level groups and cheese samples as fixed effects. The difference in milk SCC levels resulted in changes in cheese texture, being the high SCC cheeses more grainy, tough and screechy. The low SCC samples were perceived as more moist and greasy and showed a more homogeneous colour. In addition, they had a less salty and acidic taste. With regard to the flavour and odour attributes, they were not affected by the SCC level of the milk. The analysis performed by the electronic nose confirmed the results obtained by the panel. Overall acceptability was higher for the low SCC cheeses (5.46 vs 3.16 on a 9-point unstructured scale). In conclusion, results of this study indicated that SCC in milk is a major factor that can negatively affect the sensory characteristics of Pecorino cheese over 60 days of ripening.

67T

Feeding system effect on Amiata donkey's milk flavours

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Ten animals were assigned to two groups consisting of 5 each one, homogenous for days of lactation (90-146 days). One group received a basic forage diet with access to pasture (FO) while the other group was stabled and fed with forage and concentrate (CO) according to NRC requirements for lactating donkeys. The trial lasted 60 days. Every 15 days the donkeys were milked. On each sample of milk, three times replicated, the quali-quantitative aromatic profile was investigated by SPME-GC/MS and the results, expressed as percentages of the relative peak areas, were submitted to ANCOVA using the following model: $y_{ij} = \mu + a_i + b \cdot x_{ij} + e_{ij}$; where a_i = effect of diet, x_{ij} = days of lactation. The most representative compounds of the aromatic profile of the feeds were hydrocarbons and aldehydes. In the milk, 42 volatile compounds were identified and quantified. The most abundant desorbed volatiles were acids which showed significant differences ($P=0.005$) between FO (80.07%) and CO (60.51%) groups, followed by ketones (FO 4.38%; CO 10.44%, $P=0.156$), hydrocarbons (FO 3.21%; CO 10.19%, $P=0.111$), aldehydes (FO 1.96%; CO 2.94%, $P=0.354$), esters (FO 1.32%; CO 1.91%, $P=0.499$) and alcohols (FO 0.22%; CO 0.21%, $P=0.904$). In both groups, caprylic acid (FO 34.01%; CO 24.98%, $P=0.008$) and caprynic acid (FO 40.56%; CO 30.64%, $P=0.009$) were the most representative compounds. The m-cymene, identified only in the milk of FO group (0.17%), could represent a tracing compound of the feeding system. Data obtained should be supported by further studies based on the sensorial analysis to assess the olfactory threshold of some volatile compounds.

68T

Milk production of Sarda ewes fed chestnut and quebracho tannins

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To determine the effects of chestnut and quebracho tannins inclusion in the diet on milk production and quality of grazing dairy sheep, a four week trial was carried out in 48 mid-lactation Sarda ewes (16 per experimental group). All animals were fed *Medicago polymorpha* pasture (20.1% dry matter, DM; 41.5% NDF, 33.1% ADF, 18.3% CP, on a DM basis) and a supplementation of 600 g/d of concentrate. The composition of the concentrate was 88.4% DM; 23.7% NDF, 10.6% ADF, 18.8% CP, on a DM basis, for the control group, with the inclusion of a 6.6% (on a DM basis) of commercial chestnut and quebracho hydrolyzable tannins for chestnut and quebracho groups, respectively. Daily milk production was recorded and milk samples were collected weekly for analysis of fat, protein, SCC and urea. Pasture samples were collected weekly for

composition determination. Data were analyzed using a mixed model (MIXED procedure, SAS 9.1) including tannin type, sampling and their interaction as fixed factors, and the animal as random factor. The interaction was not significant. The inclusion of chestnut or quebracho tannins in the diet did not influence milk yield (1327 g/d, 1295 g/d and 1355 g/d for control, chestnut and quebracho groups, respectively), which fell linearly throughout the period without differences between groups. Milk fat and protein content were not influenced by diet. The SCC was lowered by the inclusion of tannins in the diet, with values of log₁₀ SCC (x 1000/mL) of 2.41, 2.07 and 2.16 for control, chestnut and quebracho groups, respectively. Urea content did not differ between groups. In conclusion, the chestnut and quebracho tannins, at the doses used in this trial, did not modify the productive performance and was not able to improve nitrogen utilization in lactating sheep fed pasture with high-protein content. The positive effect of tannins on SCC needs further research, to verify a possible enhancement of the immune system of animals.

69T

Causes of milk fat concentration variability in dairy cows

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This work aimed to quantify the effect of several animal and dietary factors on milk fat concentration (MF) of dairy cows kept in Mediterranean climatic conditions. From December 2009 to July 2010, 54 samples of different total mixed rations (TMR) and bulk milk were collected from 26 randomly chosen dairy farms of Arborea (Sardinia, Italy). Daily herd DMI intake and milk yield (MY) were also recorded. TMR were analyzed for chemical composition and sieved with the Penn State Particle Separator (PSPS) to estimate their physical effective NDF (peNDF).

In the studied farms MY was 27.3±2.8 kg/cow per d (mean±SD; range 20.0-33.3 kg/cow), MF was 3.79±0.26% (range 2.88-4.29%), dietary NDF was 37±2.6% of DM (range 32.2-43.9%), peNDF was 31.1±3.0% of DM (range 24.8-38.4%). Despite this large variability, no significant associations between MF and NDF, peNDF or DM in the PSPS sieves were found. MF was negatively related with the DM of TMR ($r=-0.55$; $P<0.001$) and positively associated with the concentration of silage in the ration ($r=0.30$; $P<0.05$). MF was lower in hot than in cold seasons ($P=0.03$). A multivariate factor analysis, which excluded MF, was applied to the dataset and 6 factors were extracted. They denoted: F1) diet energy content (linked to TDNm, NEL3m, apparent digestibility, ADL, fat in TMR), F2) forage to concentrate ratio (linked to starch, NFC, NDF, ADF), F3) silage effects (linked to middle and lower PSPS fractions, silage in diet, DM of TMR), F4) cow performances (linked to MY, DMI,

upper PSPS fraction), F5) diet protein balance (related to CP and bottom pan of the PSPS) and F6) season effects (linked to cold and hot season). Silage and season effects were significantly correlated with MF ($r = 0.40$, $P < 0.01$ for F3; $r = -0.33$, $P < 0.05$, for F6). F3 was positively related to MF, with a similar trend both in hot and cold seasons, highlighting the positive effects of silages on MF. The results showed that in many instances other factors than peNDF affect MF of dairy cows.

70T

Delayed response to heat stress in dairy cows

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During summer, dairy cows in Sardinia (Italy) have marked declines of milk production. Thus, this work aimed to quantify the importance and the effect over time of the main meteorological variables usually involved in the heat stress of dairy cows in Mediterranean climatic conditions. From June to September 2009 the data related to daily herd consistency, feed supplied and refused, and milk yield (MY) were obtained from a farm management software (Ecostalla®, J-Service, Arborea, Italy) currently used in one farm of Arborea (OR, Sardinia, Italy). During the same period, mean air temperature (T_m) and relative humidity (RH) were recorded, 2 m above the feed bunk, every 2 hours, and the temperature-humidity index (THI) was calculated in °F (Kliber, 1964) and converted in °C. The daily mean values of recorded variables (range within parenthesis) were: number of lactating cows 161±0.5; DMI 20.2±0.1 (18.3-22.5) kg/cow per d; MY 26.2±0.1 (23.9-28.0) L/cow per d; T_m 24.9±2.0 °C (20.7-31.7); RH 67.1±7.6% (38.6-77.8); THI 22.9±1.6 °C (19.8-25.9). DMI was negatively correlated with T_m (-0.38; $P < 0.001$) and THI (-0.44; $P < 0.001$). MY was poorly associated with the same day weather variables. Correlations were higher when DMI or MY were associated to previous days T_m and THI. The highest correlation were observed with a delay of 3 days for DMI ($r = -0.56$ and -0.61 with T_m and THI, respectively; $P < 0.001$), and with a delay of 5 days for MY ($r = -0.58$ and -0.63 ; for T_m and THI respectively; $P < 0.001$). Estimated delayed stress was equal to -0.419 kg of DMI/cow per d and -0.391 kg of MY/cow per d per increased unit of THI above the threshold of 21 °C (70 °F). The RH was significant only combined with T_m in the THI. The MY on a certain day was best predicted as $MY (L/cow per d) = 22.2 - 0.170 \times THI_{-5} (°C) + 0.390 \times SSI_{-2} (kg/cow per d)$ ($r^2 = 52.7$), where the subscripts -5 and -2 indicate the days before MY. This study suggest that delayed MY losses due to heat stress can be minimized if proper actions are taken as soon as THI increases above the threshold.

71T

Effect of lysozyme and ripening time on *in vitro* Ca digestibility of Grana Padano DOP cheese

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High Ca content is one of the most important characteristic of dairy products. According to some authors dairy products have a better Ca digestibility compared to plant foods and this characteristic has been related to the inclusion of Ca in the caseinophosphopeptides (CPP). Because ripening time and lysozyme can affect peptide pattern we decided to study the effect of these two factors on *in vitro* Ca digestibility. Twenty samples of Grana Padano produced with lysozyme and 17 samples produced without lysozyme (total 37 samples), aged between 11 and 25 months were obtained from 2 different cheese-factories. *In vitro* Ca digestibility (dCa) was determined using the methods of Perales et al (J Agric Food Chem, 2005, 53: 3721). Molecular weight (MW) of peptides was determined by exclusion chromatography and CPP were determined by HPLC-MS. The dCa (%) at ripening time of 12, 15-20 and > 20 months were: 71.75, 76.10, 86.04 for Grana Padano and 73.21, 63.70, 62.59 for lysozyme-free cheese. In Grana Padano dCa and aging were positively related ($r^2 = 0.27$; $P < 0.05$). Since Ca is almost entirely associated with caseins, the difference in dCa could be due to a different proteolysis mediated by the presence of lysozyme. In the range of MW from 1000 to 2500 D, we observed an higher number of Ca-binding sequences in lysozyme containing cheese compared to Grana Padano and this fact could explain the different result. Furthermore dCa in lysozyme containing cheese was positively correlated to the concentration of peptides having MW in the range 1000-1500 D and 1500-2500 D. A larger number of cheese samples is necessary in order to better describe the effect of ripening and lysozyme addition on Ca digestibility, however our results suggest that the use of lysozyme in cheese-making can affect peptide pattern of cheese and dCa, this could be of nutritional relevance in people with high Ca requirements such as elderly people or pregnant women.

72T

Effect of parity and day of lactation on cell content in colostrum and milk of dairy cows

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Milk contains cells with important immune-related roles. In particular, it has been recently observed that mammary epithelial cells secrete a range of immune effector molecules. The study of these cells offers great potential to improve milk positive traits for consumer health. Therefore, the aim of this research was to evaluate the presence of epithelial cells in milk of healthy cows, in different stages of lactation. Fourteen cows, 7 at 1st and 7 at 2nd or 3rd calving, were considered. On day 0, 1, 3, 7, 30, 60 and 90 of lactation, colostrum and milk samples were taken. Each sample, obtained mixing morning and evening milkings, was tested for protein, fat, lactose, casein, urea, SCC (somatic cell count) and TBC (total bacterial count). Colostrum and milk somatic cells were isolated and immunocytochemically stained to identify epithelial cells, using an anti-cytokeratin antibody. After inspection by optical microscopy, the cytological slides were classified for increasing estimated percentage of epithelial cells over the total visible cells. Data were evaluated by ANOVA or χ^2 test. Results indicated that in most of the samples of the first week after calving the intact epithelial cells were very rare. By contrast, on days 30, 60 and 90, in many slides the epithelial cells, although generally less abundant than leukocytes, were a large part of the total cells. However, on days 0 and 1, high percentages of epithelial cells were more frequently present in colostrum of multiparous cows than primiparous ones ($P < 0.05$). Furthermore, at parturition the total SCC was higher in the primiparous than multiparous cows ($P < 0.01$), even if on days 0, 60 and 90 the primiparous cows had lower TBC ($P = 0.07$) than the multiparous ones. Results suggest that milk total cell content is not merely a marker of mammary inflammation state and, like the content of epithelial cells, it may be influenced by different factors, including parity and lactation stages.

73T

Content of odd- and branched-chain fatty acids in milk and cheese of different origin

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The interest in odd- and branched-chain fatty acids (OBCFA) is increasing in the animal nutrition field due their potential use as diagnostic tools of rumen function. In addition, recent research

showed their potential anti-cancer activity. As a consequence the attention on these compounds is also growing in the dairy field. The main OBCFA in cow milk are those having 15 and 17 carbon atoms and isomers of C14, C15, C16 and C17 acids, which are derived mainly from the rumen microflora, being present in the bacterial membrane lipids. The animal diet has an influence on the amount of these fatty acids in milk fat, since the feeding can affect the rumen environment. In addition small amounts of these acids can derive from the *de novo* synthesis in the mammary gland. In our research the fatty acid composition of milk and cheese samples deriving from animal feeding both on mountain pasture in the Alpine area and on an intensive breeding system was studied. Moreover, within the mountain pasture, the effect of different alpine vegetation types and levels of supplementation were investigated. Fatty acids were determined as methyl esters and analyzed by gas chromatography using a 100 m capillary column. From the statistical evaluation of the data, interesting differences were observed in the fatty acid composition according to the type of feeding adopted. In particular the different vegetation type in one of the Alpine regions studied seemed to significantly affect the OBCFA composition of cheeses, thus the samples could be discriminated according to the pasture.

74T

Development of a real-time PCR method for quantitative detection of *Clostridium tyrobutyricum* in milk and dairy products

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Clostridium tyrobutyricum is an anaerobic sporeforming bacterium recognized as responsible for late blowing defect in hard and semi-hard cheeses. The spores survive during the long ripening period, and can germinate when favorable conditions are reached. The method to detect *Cl. tyrobutyricum* spores is based on MPN counting and confirmation of lactate fermentation capacity of cells from gas-positive tubes. This method is time-consuming and do not allow the enumeration of vegetative forms. DNA amplification-based methods may allow to overcome these problems, and the quantification by real time PCR (qPCR) is becoming increasingly common for diagnostic purposes in food microbiology. The aim of this study has been to develop a qPCR assay for the quantitative detection of spores and vegetative forms of *Cl. tyrobutyricum* based on the amplification of species-specific region of the 16S rRNA gene. The selectivity of the assay was tested against 29 strains of *Clostridium* (belonging to 9 different species) and 17 strains of 15 non-*Clostridia* species. The qPCR was inclusive for *Cl. tyrobutyricum* (15 strains tested) and 100% exclusive for non-target bacteria. The limit of sensitivity, i.e. the highest dilution

able to give a positive amplification, was around 2 log cells/ml. Before nucleic acid extraction, pre-treatments of raw milk and cheese samples were optimized to recover and concentrate cells and spores. The quantification resulted linear ($R^2 > 0.9985$) over a 6-log dynamic range with 3 different substrates, broth, milk, and cheese. The relative accuracy (R%), i.e. the agreement between log cfu/g values from qPCR and those of plating method, ranged between 89-99% in broth, 78-90% in milk, and 89-94% in cheese. The developed qPCR method could be of potential application in specific detection and accurate enumeration of *Cl. tyrobutyricum*, both spores and vegetative cells, in raw milk and cheeses.

75T

Capillary electrophoresis analysis of sialyloligosaccharides in milk of different species

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Sialyloligosaccharides (oligosaccharides containing one or more residues of sialic acid) are relevant components of human milk, which have been quite well studied for their prebiotic effect and their capacity in stimulating the immune system. Since oligosaccharides from milk of non-human mammals received so far less attention, the aim of this work was the application of capillary electrophoresis (CE) for the analysis of sialyloligosaccharides in milk of different species, to possibly identify potential sources of oligosaccharides to use as health promoting ingredients in functional foods. Mare milk (3 samples), ass milk (3 samples), goat milk (2 samples) and cow milk (10 samples), at different stages of lactation, were analysed in comparison to 2 samples of human milk. Samples were frozen at -20°C immediately after collection, thawed over-night at 4°C before analysis, defatted and deproteinized. A recent CE technique using the MEKC mode with direct detection at 200 nm was applied to resolve and quantify 3 of the most dominant sialyloligosaccharides of human milk. Commercial standards of 3-sialyllactose (3-SL), 6-sialyllactose (6-SL) and disialyllacto-N-tetraose (DSLNT) from 39 to 2500 µg/mL were used to create linear calibration curves (R^2 from 0.9967 to 0.9997), with coefficients of variation from 3 to 10%. Analysis of non-human milk samples confirmed differences among species, individuals and between stages of lactation: DSLNT, which was the most abundant compound in human milk (455-805 µg/mL) was missing in most of the samples. In most cases 3-SL showed to be the most concentrated of the analytes we quantified, with values ranging from 12 to 77 µg/mL.

76T

Effects of different diets on fatty acid profile of sheep milk and ricotta covered by the protected origin denomination "Ricotta Romana"

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The aim of the present study was to compare the effects of a pasture based diet to a standard diet and a diet supplemented with extruded linseed, on the fatty acid (FA) profile of sheep milk and its transfer to "Ricotta Romana POD". Immediately after lambing, 3 groups of 12 Comisana sheep, homogeneous for age and parity, were randomly allocated to one of 3 following dietary treatments: a control diet (C), an isoenergetic and isoproteic diet supplemented with 6% extruded linseed (E) and a pasture diet (P). Sheep were milked twice a day for 12 days after lambs slaughtering (30 d). Bulk tank milk from each group and ricotta obtained from the same milk were daily sampled and subsequently analyzed to determine fatty acid (FA) composition. Results, after testing for normal distribution, were processed by ANOVA considering the diet as source of variation. Milk fat content was not significantly affected by the different diets. Total saturated FA (SFA) were significantly higher in C than in E and P milk ($P < 0.001$) while an increase in milk polyunsaturated FA (PUFA) was evident in E group ($P < 0.001$). Particularly, the proportion of linolenic (C18:3) acid was significantly higher ($P < 0.001$) in the E group than C while P sheep ranged at an intermediate level. Conversely, the milk of the pastured sheep showed a significantly greater presence of CLA (C18:2 cis-9 trans-11), anyhow higher in E group than C. The fatty acids in the ricotta reflect their proportion in milk with a significant greater amount of SFA in C than in E and P groups ($P < 0.001$). The highest concentration of n-3 fatty acids in E and P groups resulted in a significant reduction of n-6/n-3 ratio. Rumenic acid content was significantly influenced by pasture and, even less, by linseed supplementation.

77T

Influence of postpartum on some haematological, biochemical and immunological parameters in Zerasca sheep

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During lactation the ewe is subject to intense variations of the metabolic and immune status but the relative studies in sheep are limited, especially in meat-type breed. This investigation aimed at studying the changes of some haematological, biochemical and immunological parameters in Zerasca ewes during this period. The study, that represent a first step of a larger project, was carried out on fifteen adult and clinically healthy Zerasca sheep, an indigenous meat-type breed. The farm was located in Zeri (MS) at an altitude of 900 meters a.s.l. Two blood samples were collected from the jugular vein using Vacutainer® test tubes; the first within four weeks from lambing and the second two months later. The blood samples were analysed for RBC, WBC, Hgb, MCV, MCH, MCHC, PCV, ALT, AST, BUN, cholesterol, total proteins, glucose, triglycerides, creatinine, NEFA, BPI, cortisol, lysozyme, CD4⁺, and CD8⁺. ANOVA was performed to test the overall significance of the differences between mean values. The postpartum phase resulted in exerting a significant effect upon the following parameters: ALT, cholesterol, glucose, NEFA, creatinine, lymphocyte and lysozyme ($P < 0.001$), BUN and CD8 ($P < 0.05$). Among these parameters, some of them exceeded the normal range at the second sampling: ALT, lysozyme and CD8⁺. NEFA decreased below the normal range limit, probably in consequence of the lactation which evidently represents a state of excessive catabolism. Lysozyme level was higher in lactating ewes indicating a momentary enhancement of the innate immunity. It can be noticed that the haematological results showed no alteration and was not influenced by the physiological state. The few available data do not allow to make conclusive assessments but it is possible to assert that no considerable changes in blood count, metabolic and immune systems of Zerasca sheep at this stage were observed.

78T

Passive immunity transfer and foal growing evolution

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Passive immunity transfer failure is the most important cause of

septicemia in the newborn foal. Good quality colostrum contains not less than 60 g Ig/L and low quality one less than 38 g/L. Levels normally in accordance with the foal protection are over 800 mg/dL. IgG serum concentration at 18-24 hours from first suckling, when colostrum is ingested under optimal conditions (1.5-2 litres within 6 hours from delivery). Aim of this work was to measure growing evolution by morphological traits, APGAR test and haemochrome evaluation in two thoroughbred foal groups from the same breeding with different serum IgG level concentration. IgG serum concentration from 74 foals at 18-24 hours from first suckling and at 21 days of age was determined by semiquantitative Elisa test kits. Morphological traits (body weight, withers height, chest and shin measurement), daily gain, APGAR test and haemochrome (RBC, WBC, Hb, PCV, PLT) were evaluated from 1 to 6 months of age and differences between A and B groups were statistically evaluated by JMP 8. In 70 foals (A group), 94.3%, IgG amounts were over 800 mg/dL, in 4 foals (B group), 5.7%, IgG levels were lower than 400 mg/dL. 1 litre colostrum per os from internal colostrum bank and parenteral immune serum (Plasmalife, Siena) were administered to B group foals. Body weight differences were statistically significant between A and B (266.48 kg *vs* 259.25 kg) at 6 months. No differences were observed between other morphological traits. 97.3% foals was APGAR test as 7-8. 2.3% was 6 (1 from B group, 0 from A group). No statistically significant differences were observed between RBC, WBC, Hb, PCV, PLT at 21 days test. The enlightenment to breeders about the incidence and high risk of passive immunization failure by foals should be intensified. At the same time we confirm that early and specific interventions as excellent colostrum supplementation and specific hyper-immune serum administration might prevent failure of immunity passive transfer.

79T

Influence of group size on behavioural and immune responses of buffalo young bulls

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From November 2009 to February 2010, twenty-one buffalo bulls were used to evaluate the effect of group size on behavioural and immune responses. Fourteen bulls (group HS) were group-housed in an indoor slatted floor pen with an outdoor paddock (3.2+3.2 m²/animal). Other 7 (group LS) were group-housed in a similar pen having the same space allowance per animal. The animals at beginning of experiment were aged 455±72 d and weighed 337±67 kg. Animals were subjected to seven sessions of instantaneous scan sampling at ~15-d intervals. Observations

were made every 10 min over a 5-h period (1000 to 1500 h). Behavioural variables were expressed as proportions of subjects observed in each category of posture and activity. In addition, rapid behaviours such as agonistic, social, and reproductive interactions, social licking, and self-grooming were recorded continuously. Phytohaemagglutinin and keyhole limpet haemocyanin were used to assess *in vivo* cellular immunity and antibody response, respectively. The immune responses of group HS were assessed only on seven animals chosen at random. Behavioural data were analysed with a two-way ANOVA (group size and period of observation). *In vivo* cellular immunity response was subjected to ANOVA with one factor (group size). Antibody response data were analysed using a linear mixed model for repeated measures. When more animals were involved in eating activity a higher number of aggressive interactions were observed compared to the period away from feeding (0.96 vs 0.56 n/animal/h; SE 0.13; $P < 0.05$). Immune responses were not affected by group size. As expected the antibody titer increased 2 weeks after the first immunisation and reached a peak 4 weeks after the first injection. It can be concluded that group size had not a detrimental effect on buffalo bull welfare.

80T

Effect of milking pre-partum training on behaviour and milk production of buffalo heifers

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The aim of this study was to investigate the effect of milking pre-partum training on behaviour, milk production and milk flow profile of buffalo heifers. The study was conducted in the Improstata farm of Campania Region. Fifteen animals aged 30 to 44 months were divided into two groups. Eight animals served as control (group C), whereas 7 animals received in the morning a pre-partum training for at least 7 days (group T). During the pre-partum training sessions, an animal was moved to the milking parlour (tandem 3×3) and left for 10 min in the milking stall, where the udder was thoroughly washed and massaged for 1 min. The massage included the stripping of the teats. Once calved, all the heifers were observed in the parlour for their first milking then on 7 occasions (3, 7, 14, 28, 42, 56 days after calving) during afternoon

milking. The behaviours, recorded from the entrance in the milking stall to the removal of milking cluster, were step (foot lifted less than 15 cm off of the ground) and kick (foot raised above 15 cm off of the ground, even if a clear kick was not visible). Milk flow profiles were measured with electronic mobile milk flow meters. Milk samples were also collected to assess milk quality (CFU, SCC, lactose, protein, and fat). All variables were analysed with a linear mixed model for repeated measures. Animals of group T performed a lower number of steps (0.08 ± 0.03 vs 0.20 ± 0.03 n/min; $P < 0.01$) and kicks (0.13 ± 0.04 vs 0.26 ± 0.03 n/min; $P < 0.01$) than control animals. A reduction of number of kicks was observed as the lactation proceeded ($P < 0.001$). Milk quality, milk production and milk flow profiles were not affected by treatment. It can be concluded that a milking pre-partum training would reduce the level of fear in buffalo heifers.

81T

Veal calves health status in large groups fed with automatic milk feeding device

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To analyze the main pathologic syndromes affecting veal calves reared in large groups using automatic calf feeders, a trial was conducted on 138 male calves. Calves arrived in 3 week on March-April, aging 23.2 days on average, and were reared until September, aging 176.5 days on average. During the trial they were housed in 6 groups of 20-25 each, in straw bedded pens of 50.4 m² in the same stable. Climatic parameters (temperature: mean 19.5°C, range 5.6÷32.9°C; relative humidity: mean 74.2%, range 36.1÷100.0%; rainfall: mean 41.2 mm, range 0.0÷63.4 mm) and health status were controlled daily, so as any sanitary treatment required. At arrival, all calves were preventively treated with broad-spectrum antibiotics for 5 days and vaccinated for several respiratory diseases. The individual treatments (days) needed during the trial were encoded according the pathologic syndrome in three classes: respiratory (n=349), enteric (n=43), and others (n=109), and their distribution during the trial was correlated (Spearman) every 2 weeks to climatic parameters and calves age. Respiratory treatments are negatively correlated to maximum humidity ($\rho = -0.69$; $P \leq 0.05$) and to calves age ($\rho = -0.69$; $P \leq 0.05$), and positively to rainfall ($\rho = 0.79$; $P \leq 0.01$), while enteric treatments are negatively correlated only to mean humidity ($\rho = -0.58$; $P \leq 0.05$), and other kind of treatments are positively correlated to all temperature values ($\rho = 0.62 \div 0.65$; $P \leq 0.05$) and negatively to rainfall ($\rho = -0.65$; $P \leq 0.05$). It should be outlined that during the whole trial 82 calves (59.4%) needed no treatments and the mortality rate was 6.5% (9 calves), while in the first 6 weeks from the arrival the mortality rate was 77.8% of total (7 calves) and the calves were treated at 66.5% for respiratory, at 67.4% for enteric, and at 7.3% for other syndromes.

82T

Veal calves rearing performances in large groups fed with automatic milk feeding device

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To test the possibility of rearing veal calves in large groups using automatic feeders, a trial was conducted from May to September on 130 Friesian male calves, according to the latest EU suggestion on cattle welfare. The calves were housed in 6 groups of 20-25 each, in straw bedded pens of 50.4 m² in the same stable, and reared for about 150 days. In each pen, 1 automatic milk dispenser, 4 m of linear manger for dried maize silage (as fibre feed) and 2 drinking troughs were available 24 h/d, to ensure feed and water availability. To monitor the rearing performances calves were weighted every 4 weeks and measured at the beginning and the end of the period, while the feed consumption was recorded daily, and data variability was studied. At starting, calves had an age of 30.2±7.9 d, a live weight of 51.3±6.7 kg, a withers height of 81.5±2.4 cm, a body length of 76.9±3.5 cm, a chest width of 16.3±1.3 cm. At slaughter, they reached an age of 183.5±11.2 d, a weight of 195.9±26.9 kg, a withers height of 103.4±3.3 cm, a body length of 109.3±4.2 cm, a chest width of 26.3±2.0 cm. During the trial, feed consumption was 3500±11700 kcal/head/d, satisfied with milk powder (800±2900 g/head/d in 4÷16 L of hot water) and enriched dried maize silage (200±700 g/head/d), with a global consumption of 32,275 kg of milk powder (1.41 UFV/kg DM) and 14,275 kg of maize (0.65 UFV/kg DM). In the whole period, calves' ADG was 0.94±0.15 kg/d and FCR was 2.10 kg DM/kg LW, while the live measures gain was 251.5±44.5% for weight, 27.0±3.7% for withers height, 42.3±7.5% for body length, 63.0±15.7% for chest width, showing allometric growth coefficients of 0.92, 0.17, 1.47, and 0.31 respectively ($P \leq 0.0001$), that could be considered good performances for such kind of animals, showing the feasibility to use automatic calf feeders for large groups of animals.

83T

Economic assessment of welfare in dairy cattle farmsMartino Cassandro¹, Mauro Penasa¹, Alessandro Calliman², Franco Pietrobon², Paolo Fent², Roberto Santomaso²¹*Dipartimento di Scienze Animali, Università di Padova, Italy*²*Associazione Provinciale Allevatori di Treviso, Lancenigo di Villorba, Italy*³*Associazione Regionale Allevatori del Veneto, Padova, Italy*
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Aim of this study was to investigate the impact of welfare on the economic outcome per cow and per lactation (ECOW). A total of 138 dairy farms located in Treviso province were visited once in

2010 and scored by 4 technicians for several welfare parameters using a check list. The check list included information on characteristics of the stall, watering, floor, lighting, ventilation, milking and management. Each parameter was evaluated using a five-point scale and an overall welfare index (WI) was then derived. An analysis of variance was performed on ECOW accounting for fixed effects of technician, type of barn (TB: tie-stall or free-stall), average estimated breeding value of cows for lactation milk yield within the herd (EBVL) and WI. The last two factors were included as covariates. The average ECOW was €594±317. The model explained 40% of the variability of the trait with a root mean square error of €252. Results from analysis of variance showed that ECOW was influenced by TB ($P < 0.06$), EBVL ($P < 0.001$) and WI ($P < 0.01$), while the effect of technician was not statistically significant ($P = 0.55$), suggesting that the technicians were trained properly. The increase of one unit of EBVL and WI resulted in a decrease of ECOW of €0.49 and €10.44, respectively. The check list is a feasible and easy method to assess welfare in dairy herds and results indicate that welfare has an economic impact on outcome per cow and lactation.

84T

Behaviour expressed by grazing native beef cows

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In order to evaluate the degree of adaptability of three native cattle breeds to the marginal pastures of southern Italy, the diurnal grazing behavioural pattern was monitored on 84 multiparous Podolian, Chianina and Romagnola cows (28 per breed, 3 to 12 years of age, 42 suckling and 42 non-suckling) all belonging to the same herd. From August to October 2009 ten sessions of nine hours of behavioural observations were performed, from 07.00 to 19.00 h, through continuous focal animal recording. Every hour, bite rate was recorded and bite mass and botanical preferences were determined on hand-plucked samples. Chianina cows selected proportionally more percent legumes ($P < 0.01$) and less percent ferns ($P < 0.05$) than Romagnola and Podolian animals. Due to low genetic diversity and allomimetic behaviour, no marked differences were observed among breeds in terms of activity budget, ingestive behaviour and chemical composition of the diet as assessed through hand plucking. Animals spent most of their time on grazing, followed by walking, whereas less time was spent lying, which was more often expressed at midday ($P < 0.01$). Based on the chemical composition of hand plucked samples, the minimum ingestion levels needed to cover maintenance and productive requirements of energy and protein (12.60 – 15.59 and 5.47 – 20.93 kg DM, respectively) were roughly estimated to be compatible with the potential intake of the animals. Vocalizations were not

influenced by breed but were significantly more frequent in cows with calf than in non-suckling animals (1.48 ± 0.33 vs 0.03 ± 0.33 , respectively; $P < 0.001$). These results along with the low levels of agonistic interactions occurring between herd-mates (less than 0.65 per h) suggest that extensive systems based on grazing allow social stability, expression of species – specific behaviours and fulfilment of maintenance and productive requirements of cows from native breeds.

85T

Effect of diet and genotype at *CSN1S1* locus on the oxidative stress in lactating goats

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Twenty-eight Girgentana goats were selected on the basis of their *CSN1S1* genotype as follows: 14 goats homozygous for strong (AA) alleles and 14 goats heterozygous (AF). The goats were used in a 2 x 4 factorial arrangement of treatments, with the two genotypes (G) and four diets (D). The diets were: a pelleted alfalfa hay (100H), a complete feed including alfalfa hay at 65% (65H), fresh sulla (*Hedysarum coronarium* L.) forage (100S) and fresh sulla plus barley (65S). Each trial consisted of 15d for adaptation and 8d for sample collection, during which the goats received the scheduled diet ad libitum. Blood samples were collected at the end of pre-experimental and experimental period. Plasma content of Reactive Oxygen Metabolites (ROMs), biological antioxidant potential (BAP) and α -tocopherol were measured by colorimetric assay and HPLC, respectively. Data were analysed by ANOVA including effect of G, D and interaction G x D. There was no significant effect of G and interaction G x D on all parameters. Diet significantly influenced ROMs, BAP and α -tocopherol. The ROMs concentration was significantly lower ($P < 0.05$) in goats that received fresh forage 100S and 65S (4.20 and 4.53 lnUCarr) compared to goat groups that received hay 100H and 65H (5.24 and 5.20 lnUCarr). The BAP was higher ($P < 0.05$) in 100S, 65S and 65H (respectively 8.01, 7.99 and 7.95 ln μ Eq/L) than in 100H (7.66 ln μ Eq/L). Plasma α -tocopherol was significantly higher in 100S and 65S diets compared with 100H and 65H diets (respectively 2.36 and 2.17 vs 1.79 and 1.81 ln μ mol/L; $P < 0.03$). A negative correlation between ROMs and α -tocopherol concentration was observed ($r = -0.80$; $P < 0.05$). On the contrary, positive correlations between α -tocopherol and BAP plasma content ($r = 0.31$; $P < 0.02$) and milk production ($r = 0.28$; $P < 0.03$) were found. This study has

identified that diet (fresh forage vs dry diet) rather than genotype has a more pronounced effect on oxidative stress.

Acknowledgement

The research was funded by MIUR, project 2007B4JBWN_003

86T

Metabolic biomarkers for heat stress evaluation in poultry: preliminary results

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In the meat production and consumption context, poultry meat plays an important role. Since a few years, the commercial poultry farms welfare has been taken into consideration. In intensive condition, high environmental temperature could determine animal stress. Aim of this study was to evaluate some metabolic parameters linked to heat stress in two poultry genetic types. One hundred and twenty Ross (R) and 120 Naked neck (NN), were divided in 2 groups: control, in conventional housing condition and heat stress group reared at 34°C (21d before slaughtering). Before slaughter blood samples were taken to evaluate plasma concentration of the reactive species of oxygen (ROS), total antioxidant capacity (PAO) and heterophils-lymphocytes ratio (H/L). After slaughter half *Pectoralis major* muscle was excised from carcasses to assess lipid oxidation level (TBARs) and total antioxidant content (TAC = γ tocopherol + α tocopherol + retinol + lutein + zeaxanthin). *In vivo* oxidative status was affected by heat stress by an increasing of ROS levels only in NN bird (0.33 vs 0.45 mM H₂O₂); this was confirmed by the significant ($P < 0.05$) increase of H/L values (0.89 vs 1.23). R birds showed an increase of PAO (136.42 vs 143.15 μ M HClO mL⁻¹) that probably counteracted the ROS increase, whereas no effect was observed in NN. In muscle of stressed R birds a significant ($P < 0.05$) reduction of TAC (507.07 vs 337.86 ng g⁻¹) was observed, whereas no significant change in TBARs was found. NN muscle showed, as for *in vivo* oxidative status, the worst condition (92.08 vs 202.03 ng g⁻¹). In conclusion on the basis of these preliminary results, it seems that R birds could better adapt to heat stress respect NN ones making metabolic changes in their antioxidant mechanisms.

Acknowledgement

Research supported by PRIN 2008 prot FN93B3_002

87T

Influence of environmental conditions on growth of weaned piglets

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To evaluate the effect of temperature and humidity, and housing conditions on productive performance of piglets during the post-weaning period, 120 male piglets (Pietrain × Pic), with an age between 28 and 84 days, were assigned to groups of 30 each, in two farms (A and B) and two different seasons, winter and spring. All the groups were fed with the same diet. Farm A had lower housing conditions in comparison with B. Each piglet was weighed throughout the experiment, and average daily weight gain (ADWG) was calculated as the ratio of the difference of weight between two successive weighing and the number of days between the weighing. Feed consumption, and temperature and relative humidity for calculating temperature humidity index (THI) were daily registered. Data regarding ADWG and feed consumption were examined by means of a two-way ANOVA, including the farm and the season as random factors. Maximum THI was higher in farm B, where it reached uncomfortable values during the spring (78 vs 76) while during the winter it did not exceed the threshold for heat stress (72 vs 69). Bacterial enteritis and respiratory syndrome had the same incidence in the two farms. Eight piglets (4 in A and 4 in B; 5 in winter and 3 in spring) died by the age of 49 days, because of bacterial enteritis. Furthermore, ANOVA evidenced higher ADWG in farm B than in A (560 vs 440 g/day; $P \leq 0.001$), and in spring than in winter (530 vs 460 g/day; $P \leq 0.05$). Also feed consumption was higher in farm B (940 vs 720 g/day; $P \leq 0.001$), and in spring (890 vs 770 g/day; $P \leq 0.001$). Feed-conversion ratio was not different between the farms and the seasons. In conclusion, in growing farms for piglets, general housing conditions seems to be more significant than temperature and humidity in affecting growth productive performance.

88T

Automatic measurement of rumination activity in lactating cows during summer season

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Available data suggest that rumination is depressed during heat stress. The aim of our trial was to evaluate the effect of high tem-

peratures on rumination time (RT) using innovative system. The Hi-Tag rumination monitoring system used in this study consists of rumination loggers positioned on the neck, stationary readers, and software for processing the electronic records. The system summarizes the RT in 2-h intervals. This study used 60 Italian Friesian cows in mid-lactation, raised in a private herd of 286 dairy cows, and fed TMR once daily. Data were collected during summer and the total daily RT, the daytime and nighttime RT were calculated. Daily minimum, mean and maximum temperature were recorded as well as individual daily milk yield (MY). The data were processed using mixed procedure for repeated measures as well as linear and quadratic regressions using RT as dependent variable and daily temperature, MY and DIM. During the trial two hotter periods (HP) were observed. During HP the daily maximum temperature ranged between 32.8 to 38.1° C and the daily minimum temperature ranged between 19.6 to 24° C. Overall total daily RT was 516±87 min d⁻¹ (281±52 and 234±48 min d⁻¹ during nighttime and daytime, respectively). During HP the total daily RT and the daytime RT were significantly lower than the values observed before and after those periods. Overall MY was 21±7 kg d⁻¹ and daily MY was correlated with the total daily RT ($r=0.30$; $P<0.001$). A quadratic effect of daily temperature on total daily RT and on daytime was observed, but not on nighttime RT. The temperature, MY and DIM explain the 10% of the RT variability, highlighting that the decline of the RT was non considerable till to a minimum daily temperature of 18°C and to a maximum daily temperature of 28°C. In conclusion high temperatures impact negatively on RT, thus this effect has to be considered during summer to counteract the greater risk of digestive upsets.

89T

Attenuation of inflammatory response phenomena in periparturient dairy cows by administration of $\omega 3$ fatty acids

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High-genetic dairy cows undergo a sudden and marked metabolic challenge around calving, often coming with an inflammatory-like state triggering the release of many lipophilic mediators (e.g. eicosanoids) more or less pro-inflammatory. As these derive from some enzymatic pathways acting on polyunsaturated fatty acids (FA), it may be possible to modulate their synthesis by altering the ratio of main precursors ($\omega 3/\omega 6$ FA). This research aimed to study the consequences of $\omega 3$ FA diet fortification around calving (2 weeks before and after it) on inflammatory response and performances. Ten cows received 22 g/d of $\omega 3$ (OPT), while 10 were used as control (CTR). Cows were frequently monitored (health status,

body condition score = BCS, blood, milk yield) for the whole transition period. A wide plasma profile, including positive and negative acute phase reactants and FA, was evaluated. Statistical analysis was carried out by repeated measures variance test. Milk production was good in both groups, but OPT *vs* CTR cows had a mild post-calving lipomobilization, confirmed by the smaller BCS drop and lower post-partum NEFA and BHB (0.57 *vs* 1.62 mmol/L at 1st wk; $P < 0.1$) levels. All cows underwent an inflammatory condition around calving, but OPT showed a less strong response, as suggested by lower levels of haptoglobin and bilirubin and higher ones of albumin and retinol. OPT *vs* CTR increased plasma concentration of $\omega 3$ FA during treatment (EPA 1.08 *vs* 0.53% and DHA 0.31 *vs* 0.08% of total FA for OPT and CTR respectively; $P < 0.01$). Since $\omega 3$ FA are the main replacers of arachidonic acid in membrane phospholipids, the increased levels of blood $\omega 3$ FA observed in OPT cows may have cut the formation of arachidonic-derivatives (more pro-inflammatory), contrasting the inflammation start. Hence, $\omega 3$ FA administration during the transition period seems to attenuate subclinical inflammations and, consequently, to improve the energy balance.

90T

Proinflammatory cytokines and acute phase response profiles in periparturient dairy cows

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Pro-inflammatory cytokines (PIC: IL-1, IL-6 and TNF- α) raise body temperature, induce anorexia, increase catabolism and cause several endocrine and metabolic changes. PIC play key roles in the regulation of acute phase response (APR). However, in transition cows the relation between inflammation and health conditions and the link between PIC, inflammation start, APR, liver function and metabolic state need to be further investigated. Therefore, 10 multiparous cows were studied 4 wk before and after calving. Cows were daily monitored for health status, feed intake (DMI), milk yield, rectal temperature; body weight and BCS were measured every 2 wk. Blood was also frequently sampled and assayed for a wide metabolic and inflammatory (including IL-1 β and IL-6; ELISA methods) profile. One cow died 3 days after calving and was evaluated separately. The remaining 9 were retrospectively classified into 2 groups according to IL-6 plasma values peak: less (LO-IL, 6 cows) or greater (UP-IL, 3 cows) than 650 pg/ml. Data were analyzed by the MIXED repeated procedure of SAS. Generally, IL-1 β and IL-6 were already higher 2-4 wk before calving, in absence of clinical symptoms. The died cow had the highest levels of IL-6 (~2000 pg/ml). Both PIC tend to decrease thereafter, showing a short-lived increase at parturition. A rise of PIC was also noted in association with clinical events close to calving, while very low levels were observed in cows with mild ketosis. UP-IL cows suffered

more severe diseases (e.g. mastitis), had lower DMI before calving, lost more body reserves and produced less milk. Moreover, UP-IL (vs LO-IL) cows showed higher haptoglobin, bilirubin and urea and lower albumin and Zn levels in the wk before and after calving. Thus, high PIC values in dry period seem related to some subclinical events as confirmed by lower DMI as well as to critical metabolic and inflammatory conditions at calving time and thereafter (e.g. more severe diseases and APR).

91T

Lactulose test for the assessment of the intestinal permeability in ram after repeated indomethacin treatments

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Increase of the intestinal permeability (IP) can occur for local pH reduction and lipopolysaccharides (LPS) release by bacteria; it can cause local and systemic inflammation with consequences for animal health. Changes of IP can be evaluated by a non-invasive test (never used in ruminant) based on oral dosage of lactulose -usually not fermented or absorbed- and its recovery in blood. This study aimed to induce intestinal damage (ID) in rams, by challenge of indomethacin, and to monitor it using the lactulose test (LACT) and blood indices. With a change-over design, 4 rams were subjected to 4 intramuscular injections (12 hours from each other) of saline (C) or indomethacin (T). Two hours after last injection, the rams were administered orally 30 g of lactulose. Blood samples were collected daily, for analysis of a metabolic profile and every 2 hours during the LACT for lactulose recovery in blood. Data were evaluated by ANOVA using the MIXED procedure (SAS Inst.). Three out of 4 rams in T showed -at different time- a blood lactulose peak, confirming clinical symptoms of diarrhoea and blood in faeces. T rams also showed oxidative stress (e.g. reactive oxygen metabolite 17.3 *vs* 11.5 mg H₂O₂/100 mL in T and C respectively $P < 0.01$ 60 h post treatment), and inflammatory response: rise of positive acute phase proteins (e.g. haptoglobin 1.2 *vs* 0.1 g/L in T and C respectively, $P < 0.01$ 36 h post treatment) and drop of negative acute phase reactants (e.g. cholesterol, albumin and paraoxonase $P < 0.05$). A good agreement between blood inflammatory indices and lactulose levels in blood was evident: T ram with low ID also showed almost an unchanged blood profile. We were not able to establish whether these results are the consequence of mucosa inflammation or LPS translocation in bloodstream or both. To clarify these hypotheses further experiments are recommended also in other ruminants, in particular dairy cow fed highly fermentable diets.

92T

The effect of high environmental temperature on broiler welfare: a thermographic approach

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The aim of the present study was to investigate the effect of high environmental temperature on broiler welfare through a non invasive objective method. High temperature represents a production challenge in many countries with a high negative economic impact. Thermography is a non invasive technique that could be used to remotely record skin temperature at distance. Temperature assessment may be lead without interfering with animal behaviour. This research was carried out on 28 birds randomly chosen (sex ratio: 1/1), commercial Hybrid: Ross 508. Birds were reared under two different environmental temperatures: 34°C [High Temperature (HT)] and 22°C [Control Temperature (CT)], all the birds were reared in standardized condition and fed the same diets. Data were collected at 4 and 7 weeks of age. A thermocamera AVIO TVS 500 was used. Each bird temperature was recorded at 5 areas (Periocular, Wing, Shank, Foot, Second Finger). SAS statistic package was applied to data analysis: physical measurements were analysed by ANOVA (GLM), environmental temperature and measurement areas were considered as sources of variance. Student's t-test was applied to the calculation of the least square means difference. The recorded differences underline the birds' ability to cope with different environments and different environmental stressor levels. These results describe thermographical characteristic of birds reared under high temperature and the effect of this negative environmental characteristic on intensively reared chickens. Temperature differences of the considered areas have been underlined. The periocular area was recorded to be the hottest one, a clear vasoconstriction at foot level was recorded in CT.

93T

Effect of long-term β -agonist treatment on cAMP and corticosterone levels

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Clenbuterol is largely used by sufferers of breathing disorders as a decongestant and bronchodilator. Moreover, it is a non-steroidal anabolism enhancer and metabolism accelerator through a mechanism based on stimulation of β -receptors on the cells of target organs. Activation of β -receptors increases intracellular cAMP, that can escape into plasma. Via cAMP-dependent mechanisms, glucocorticoids act in the regulation of inflammation and carbohydrate metabolism. The aim of this work was to examine the effects of long-term dietary administration of clenbuterol on fasting and post-feeding plasma cAMP and corticosterone in rabbits, in order to ascertain if its role as anti-inflammatory and growth promoting agent is mediated by modulations of their plasma concentrations. Eighteen 2-kg male New Zealand White rabbits, divided into 2 homogenous groups, were fed a control diet or a control diet plus 1 mg/kg clenbuterol for 31 d, *ad libitum* from 18.00 h to 09.00 h. Blood was withdrawn at the end of the experimental period at 18.00 h to 09.00 h and analyzed by RIA. The data related to plasma were processed by two-way ANOVA in order to evaluate the effects of treatment and feeding state. Treatment increased the mean daily gain (+34%; $P<0.001$) and reduced feed conversion ratio (-22%; $P=0.001$). Clenbuterol did not influence post-feeding (09.00 h) nor fasting (18.00 h) plasma cAMP, but increased fasting corticosterone (26.8 vs 41.2 $\mu\text{g/l}$; $\text{SE}=6.3$, $P<0.05$). In control group plasma cAMP was correlated ($P<0.01$) to corticosterone (post-feeding $r=+0.754$; fasting $r=-0.714$), but in treated animals cAMP was correlated to corticosterone only in post-feeding state ($r=+0.67$, $P<0.05$). Results show that the long-term β -agonist treatment raised fasting plasma corticosterone and that the higher levels did not actually affect plasma cAMP. Moreover, the treatment impaired the link between plasma cAMP and corticosterone, observed during fasting in the control group.

94T

A report on parturition, physical status and survival of progeny of a bull clone

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Neonatal death and abnormalities have been reported in a significant proportion of bovine clone fetuses, as probable result of epigenetic dysregulation occurring during the reprogramming of nuclear activities. Limited data are available on whether these abnormalities can be transmitted from a bull clone to their sexually reproduced offspring. In this study, parturition, physical status and survival of progeny (n.8) of a Holstein bull clone (CLp) was monitored and compared to offspring (n.11) of corresponding Nuclear Transfer-donor bull (DONp). All calves used in the present study were obtained from Holstein heifers recipients implanted with embryos fertilized *in vitro* using frozen semen of bull or its clone. No enlarged umbilical vessels or others physical abnormalities were observed, with the exception of one case of Large Offspring Syndrome in DONp. No difference was found between CLp and DONp for gestational length that averaged 269.5 d (RMSE 6.43); gestational length showed a correlation ($R^2=0.54$) with birth weight (min 26, max 63 kg), that was 44.8 ± 2.26 and 38.6 ± 2.66 for DONp and CLp, respectively ($P=0.0939$). Calves were in proper position at birth and delivered vaginally, except for one case of anomalous presentation and two caesarean sections concerning DONp. Dystocia occurred in 4/10 DONp and 2/8 CLp ($P=0.3167$). Seven DONp and two CLp calves died due to neonatal diarrhoea and severe case of coccidiosis ($P=0.1000$). In conclusion, no remarkable differences occurred between progeny of bull clone and its conventional counterpart for condition at birth. However, it should be noted that some anomalous birth weights were observed in both groups. Reproductive technologies, as *in vitro* production embryos and embryo transfer adopted for obtaining the calves used in this study, would be a probable explanation for these cases of large size of calves at birth and associated dystocia.

95T

Critical indoor stocking density in lactating ewes reared semi-extensively in Mediterranean areas during the summer season

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The present work describes a behavioural and productive approach in order to evaluate critical stocking density in lactating dairy ewes reared semi-extensively, during summer season in the Northern area of the Mediterranean Basin. Sixty lactating ewes were subdivided into four groups of 15 heads each (two experimental groups twice replicated). All the animals were conducted on pasture from 06:00 to 18:00, and in experimental indoor areas from 20:00 to 05:00. Two groups had an outdoor area allowing 2.5 m²/head, available during night housing in the pens. The indoor stocking densities tested were: low stocking density (1.5 m²/ewe, as enacted by organic farming EU laws) with and without external paddock (respectively LDP and LD), medium stocking density (1 m²/ewe) with and without external paddock (respectively MDP and MD) and high stocking density (0.5 m²/ewe) with and without external paddock (HDP and HD). Data were submitted to two-way ANOVA for repeated measures and Tukey test, considering as independent variables stocking density, paddock availability and their binary interaction. Aggressive behaviour was the most frequent in HD group ($P<0.01$). On the contrary, the ewes of the HDP thesis did not show aggressive behaviour. Milk and fat yield were lower in the HD ewes ($P<0.01$). Moreover, in this experimental group the somatic cell count was the highest one ($P<0.01$). The presence of outdoor areas greatly reduced the negative effects of high animal density. Behavioural and productive parameters showed a high sensibility in relation to stocking density, above all in animal housed without paddock. In the Mediterranean Basin area, organic sheep farming can be conducted with a space allowance of 1 m²/head indoors associating 2.5 m²/head outdoors for lactating ewes during night, without affecting both animal welfare and production, so in force sheep organic farming European regulations could be modified.

96T

Presence of orexins and orexin 1 receptor in bovine and pig testis

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Orexins A and B (OXA and OXB) are two peptides discovered in the 1998 in the rat lateral hypothalamus and neighboring areas, in the so called feeding center. They bind two G protein-coupled receptors, orexin 1 and orexin 2 receptors. The first is highly selective for OXA, while the second shows similar affinity for both the peptides. The orexins are involved not only in the food intake but also in the regulation of sleep/wake cycle, arterial pressure, heart rate and sexual behaviour. In this work we have investigated the expression of orexins and orexin 1 receptor in the testis of Marchigiana cattle and Suino chiaro pig (Landrace and Large white crossbreed) breeds. In detail, the testis from five animals of

each species were collected in a local slaughterhouse and were processed for avidin-biotin immunohistochemical technique. We have observed, in the bovine testis, numerous OXA and OXB immunoreactive Leydig and peritubular cells. Also we identified rare OXA immunopositive Sertoli cells. Instead in the pig testis, we have detected many orexin 1 receptor immunoreactive spermatids. Literature data show few functions performed by OXA in the male genital apparatus. In the rat, OXA inhibits the production of Müllerian Inhibiting Substance and Stem Cell Factor by Sertoli cells. The first is steroidolytic factor and the second stimulates DNA synthesis in the spermatogonia and so the proliferation of germinal epithelium. Our results represent the first data regarding the presence of OXA, OXB and orexin 1 receptor in the tubular and interstitial cytotypes of the bovine and pig testis. Moreover, they provide a starting point for further investigations useful to study in deep the role of these peptides in the bovine and pig testicular steroidogenesis and spermatogenesis.

97T

Searching relationship between backtest and tonic immobility test in piglets

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In attempting to identify individual reaction patterns in pigs, the responses to backtest (BT) and Tonic Immobility Test (TIT) have been used. BT and TIT allow to classify piglets in different types of coping style (HR=High-Resisting; LR=Low-Resisting for the BT; non-TI= don't show tonic immobility and TI=showing tonic immobility for the TIT) on the basis of their reaction to restrain. HR pigs seem to adopt a (pro)active coping style whereas LR pigs seem to behave as reactive copers. Likewise, it has been proposed that TIT is one possible way of assessing whether a pig is more likely to adopt a more active or a more passive behavioural strategy in a challenging situation. There are no data concerning the relationship between TIT and BT responses in piglets; the aim of this study was to investigate this potential relationship. Sixty piglets of commercial crossbred (Landrace x Large White) reared in the same farm were examined. The BT was performed on all subjects at 10 and 17 days of age; the TIT was carried out at 9 days (20 piglets), at 13 days (20 piglets) and at 19 days (20 piglets) of age. Statistical analyses were performed by Mann-Whitney (U) and χ^2 . Results of the tests were not influenced by their temporal sequence (BT: P=0.16; TIT: P=0.58). In the BT, the duration (sec) of vocalization (P=0.02), struggling (P=0.0001), and relaxation (P=0.005) were significantly different between HR and LR piglets. Due to the differences in methodology, the duration of these behaviours were not recordable in the TIT. There was not a correspondence between the BT and TIT categories (P=0.29), not all LR showed immobility response, and on the other hand not all HR did not show it, during the TIT. This result suggests that the two tests measure reactions to different challenging stimuli and they may be seen as indicators of different types of response to restrain.

98T

Alternative animal protein sources for aquaculture: a preliminary study on nutritional traits of Mediterranean brocade (*Spodoptera littoralis* Boisduval) larvae

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In formulating diets for farmed aquatic species, the main ingredient used is fish meal (FM). Due to the growth that aquaculture is experiencing throughout the world, the increasing demand of feeds is resulting in a FM price increase that represents an economic limit for fish farming. The need to seek protein sources alternative to FM is a matter of great interest. Among others, the use of insect larvae as feed for fish represents an interesting alternative. The aim of this study was to evaluate the nutritional traits of mediterranean brocade larvae (*Spodoptera littoralis* Bois.) in view of their possible use as feedstuffs for rainbow trout. A sample of mediterranean brocade larvae (MBL) (VI instar) and one of pupae (MBP) were assayed for fat (EE), ash (ASH) and crude protein (CP) contents by conventional methods. Amino-acidic profile was obtained by HPLC on sample hydrolysates. Relative digestibility of protein (RDP) was estimated by the Lazo (1988) method. Data, expressed on DM basis, were compared with those of a commercial feed (CF). The fat content of MBL was lower than that recorded for CF (9.4% vs 13.6%) while MBP showed a very high value (30.3% DM). Ashes in MBL (7.7%) and MBP (6.7%) were lower than in the CF (10.9%). The CP content of MBL (44.3%) was slightly higher than the CF (40.0%) but lower than in MBP (59.1%). The RDP of MBL and MBP were about a half of the value observed in CF (27.2%). Both MBL and MBP showed a lower percentage of essential amino acids (AA) (35.8%, 36.8%) compared to CF (39.4%) even if the AA profiles were in line with the requirements found in literature for rainbow trout. In conclusion, these preliminary results suggest that preimaginal stages of *S. littoralis* may be of interest in fish feeding, though more exhaustive studies have to be performed to fully assess their use as alternative for fish meal.

99T

Discrimination between raised and wild European sea bass through image analysis

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This study aimed at verifying if computer image analysis could represent a good technique to discriminate between reared and wild European sea bass (*Dicentrarchus labrax*). Images were acquired from sea bass of known origin (n=47 reared and n=13 wild). Images were taken using a digital camera and standardized procedures and were analysed using ImageJ, an image-processing program. Each fish was described by 7 morphometric, 4 shape and 3 colour descriptors that were automatically measured by the software. The data resulted normally distributed and were submitted to one way-ANOVA that considered the production system (PS) as fixed effect. Linear discriminant analysis (LDA) was used as classification method to identify sea bass PS. Any morphometric parameters (i.e., area, length) were different between PS, while solidity (0.93 vs 0.91, P<0.01), mean gray value (94.7 vs 76.0, P<0.001) and median gray value (89.9 vs 72.6, P<0.001) were higher in reared if compared to wild sea bass. Solidity, the ratio between area and convex area of an object, could be possibly related to fat distribution, muscles development and growth condition. Gray values have a range between 0 (black) and 255 (white), meaning that caught sea bass was darker. Colour differences could be explained by dietary and environmental factors such as water temperature and chemical parameters. Analyzing all the 14 features using LDA method led to a 3.3% error of classification after cross-validation. Solidity, mean and median gray values resulted in a 93.3% right classification. Image analysis could be an effective tool to discriminate between reared and wild sea bass, even though further research is required to confirm its on-line application.

100T

Metabolic profile assessment in brown trout (*Salmo trutta fario*) by mid infrared spectroscopy

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The metabolic profile (MP) could be considered a set of information describing nutritional or health status. The haematic parameters depend on feeding, rearing conditions and water quality. Changes of some specific target can be used in preventive health examination. The study of Mid-Infrared spectrum (MIR - λ 400-4000 cm^{-1}) provides a wide range of information regarding organ-

ic compounds. This work showed the potential analytical application of MIR on plasma of brown trout (*Salmo trutta fario*). Samples were collected from Broodstock (B, n=24) and Fattening (F, n=29) anaesthetized fish. The MP was assessed by using a BM Hitachi 911 analyzer. The parameters analyzed were: total protein (TP), albumin (ALB), globulin (GLOB), glucose (GLUC), cholesterol (CHOL), triglycerides (TRIG), non-esterified fatty acids (NEFA), aspartate aminotransferase (AST), amylase (AMY). The spectra were collected using Thermo Avatar FT-MIR. 150 µl of plasma was distributed in a thin layer on a ZnSe surface and then dried. Partial least square regression with mathematical pre-treatments was adopted. PRESS (Prediction Residual Error Sum of Square) was used to define the factor numbers. Satisfactory performances for qualitative assessment (r^2 = coefficient of determination in cross-validation) were obtained for protein (TP, $r^2=0.81$; ALB, $r^2=0.76$; GLOB, $r^2=0.66$), CHOL ($r^2=0.78$) and TRIG ($r^2=0.71$). The Broodstock showed some parameters at paraphysiological levels due to the reproductive phase. The principal component analysis (PCA) allowed the differentiation between F and B fish (91% correctly classified). The MIR allowed a quick monitoring of stock reared as regards to main markers of MP.

101T

Effects of cooking on textural properties of meagre (*Argyrosomus regius*) fillets from two different methods of rearing (tanks and cages)

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Texture is an important attribute of fish. Producers and consumers are interested in fish texture because it influences fish processing and sensorial profile. Cooking can markedly modify texture by inducing collagen gelatinization, myofibrillar proteins denaturation, water losses and fat drippings. The aim of the current work was to describe the effects of cooking on textural properties of farmed meagre (*Argyrosomus regius*) from two different rearing systems: tanks and seawater cages. Fish were sampled in May and July from tanks (n=36) at constant temperature (about 20°C) and from seawater cage (n=36) in the farm "Il Vigneto" (Grosseto, Italy). Left and right fillets were analyzed raw and steam cooked after cooling, respectively. Textural measurements were taken with a texturometer equipped with a 200 N load cell, performing one cycle compression test on epaxial, ventral and caudal part and one cycle shear blade test on the central part. Data were analysed by PROC GLM procedure including weight, methods of rearing, sampling date, day of storage and the interaction (methods of rearing x day of storage) in the model. Fish from tank and cage weighted 1009 vs 902 g ($P<0.05$), respectively. Cooked fillets compression test values from tank and cage differed significantly ($P<0.01$): 4.10 vs 2.90 N, 3.10 vs 2.60 N and 2.97 vs 2.20 N

in epaxial, ventral and caudal part, respectively. No significant differences resulted in shear test. Both raw and cooked fillets from tank showed a higher compression force applied than those from cage and it could be explained by constant temperature of tank that generally makes fillets harder by increasing number and thinness of muscle fibres. The greater hardness values from tank were not nullified by cooking. Further analysis should be performed to investigate the relationship between rearing method and muscle development.

102T

Seafood flavor by SPME-GC/MS

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Shellfish are appreciated for the sensory properties (odour, texture, taste and appearance) and are consumed as raw, cooked or processed. Free amino-acid profile, fatty acid composition, and volatile fraction are important parameters with a strong influence on the aroma and flavour of shellfish as well as on their evolution during the refrigeration storage. Objective of this work was to select an idoneous procedure for the determination of volatile compounds that characterizes fresh bivalve molluscs (*Mytilus galloprovincialis*, *Ostrea edulis*, *Cerastoderma edule*, *Venerupis pullastra*, *Ensis ensis*) and a crustacean (*Pollicipes polymerus*), all from Ria De Vigo (Galicia, Spain). Analytes were concentrated on a fused silica fibre with carboxen-polydimethylsiloxane coating employed in headspace mode (HS-SPME) and selectively determined using gas chromatography in combination with mass spectrometry (GC-MS). After testing different incubation temperature (60-80-100 °C), incubation time (5'-15'-30') and in vial extract volume (1-3-6 mL), the best combination selected for the analysis execution was 80°C, 30', 6 mL. A saline extraction of the raw edible part of the animals was incubated to concentrate analytes. Quantification and identification of volatile compounds was performed by MS in selected ion monitoring mode (SIM) using the internal standard method. This study demonstrated that a major composition of alcohols and aldehydes characterizes the volatile profile of the analyzed samples. In particular, 2,4-octadien-1-ol and 1-penten-3-ol characterize *M. galloprovincialis*; 1,5-octadien-3-ol and 1-octen-3-one characterize *O. edulis*; 1,5-octadien-1-ol and 2,6-nonadienol characterize *C. edule*; 1,5-octadien-3-ol and 2,4-heptadienol characterize *V. pullastra*; benzaldehyde and 1-octen-3-ol characterize *E. ensis*; 9,12,15-octadecatrienol and indole characterize *P. cornucopia*.

103T

Quality and safety in oysters (*Crassostrea gigas*) and mussels (*Mytilus galloprovincialis*) reared in Orbetello lagoon (Central Italy)

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Shellfish are perishable products due to their biochemical composition. Under refrigerated storage conditions, the shelf life of these products is limited by microbiological and enzymatic spoilage. Aim of the work was the evaluation of the influence of culture site on quality and safety of oysters and mussels, during the post-harvest refrigerated storage. In this study, 145 oysters and 90 mussels, sampled in Orbetello Lagoon (Italy), at March and at September respectively, were monitored during the storage at 4°C. The shellfish were analysed at 1, 3, 7 and 10 days from the harvesting by counting of aerobic psychrotrophic bacterial, pseudomonads, coliform bacteria and *Escherichia coli*. In order to deepen the knowledge about the role of pseudomonads, the significant number of strains from oysters and mussels were isolated. Total DNA was extracted from the isolates and 16S rDNA was PCR-amplified using FD1 and RD1 primers. Amplicons were subjected to ARDRA analysis using the restriction endonuclease CfoI. Representative strains of ARDRA groups were identified by 16S rDNA sequencing. In oysters, the aerobic psychrotrophic bacterial counts was 4.5 and 6.0 log CFUg⁻¹ on the 1st and the 10th day, respectively; the pseudomonads values was 3.2 and 5.3 log CFUg⁻¹ on the 1st and the 10th day respectively. In mussels, the aerobic psychrotrophic bacterial count was 5.8 and 6.2 log CFUg⁻¹ on the 1st and the 10th day, respectively; the pseudomonads value was 4.2 and 6.2 log CFUg⁻¹ on the 1st and the 10th day respectively. The coliform number was always below the limit permitted by the Italian law and *Escherichia coli* was always undetected. 16S rDNA sequencing indicated that the analysed isolates (pseudomonads), from oysters and safety mussels, belong to *Pseudomonas spp.*. Oysters and mussels maintained appreciable quality and safety characteristics during ten days of refrigerated storage.

104T

Molecular cloning and expression analysis of genes involved in the compensatory growth of sea bass (*Dicentrarchus labrax*)Samuela Corà, Genciana Terova, Simona Rimoldi,
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There are several descriptions of compensatory growth (a phase of

accelerated growth when food levels are restored after a period of growth depression) in fish, but the mechanisms involved in such rapid recovery from fasting are still not fully understood. Such mechanisms have principally been searched for at the level of total fish growth, but only little information is available at the molecular level. Accordingly, the present study has focused on identifying candidate genes which are involved in the compensatory growth induced by fasting and subsequent refeeding in sea bass (*Dicentrarchus labrax*). In particular, we report on the molecular cloning and sequencing of genes such as $\Delta 6$ desaturase, lipin, peroxisome proliferator-activated receptor (PPAR γ) and oligopeptide transporter (PepT-1) involved in lipid and protein metabolism. We have also analyzed fasting- and refeeding -induced changes in the expression of the aforementioned genes, in different tissues, by using real-time RT-PCR quantification. In sea bass liver, 35 days without feed contributed to a significant increase in $\Delta 6$ desaturase transcript levels as compared to ad libitum fed controls, whereas recovery from fasting (21 days of refeeding) was associated with a significant decrease in $\Delta 6$ desaturase mRNA levels. The mRNA levels of lipin and PPAR γ in sea bass liver followed the same pattern: a significant increase after fasting, and a significant decrease at the end of refeeding. PepT1 was highly expressed in the proximal intestine, and fish nutritional status significantly influenced its mRNA copy number inducing a down-regulation during fasting and an up-regulation during the refeeding. In conclusion, our findings offer new information about the dietary regulation of $\Delta 6$ desaturase, lipin, PPAR γ , and PepT1 genes expression supporting their involvement in sea bass compensatory growth induced by refeeding.

105T

Effects of *postmortem* storage temperature on sea bass (*Dicentrarchus labrax*) muscle protein degradation: analysis by 2D DIGE and mass spectrometryElena Preziosa, Samuela Corà, Genciana Terova,
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Given the recognized importance of storage conditions for post-mortem deterioration of fish muscle, this study has focused on the storage temperature, which is considered the factor with the strongest impact in this process. Differences in the abundance of muscle proteins, due to biological *post mortem* processes, were studied by 2D DIGE and MS in 6 sea bass (710±157.87 g) kept at either 1°C or 18°C for 5 days. The results demonstrated that sea bass muscle proteins within the molecular weight and pI values investigated here are proteolysed to a relatively less degree as compared to *post mortem* mammalian muscle. The greatest alterations in sea bass filet protein composition can be ascribed to the 18°C *post mortem* storage, and distinct changes appear after 5

days of storage at this temperature. With respect to the myofibrillar protein myosin heavy chain, it was showed that, in sea bass muscle, this protein may degrade even at very low temperatures of storage. The results of this study offer new knowledge on the protein changes in fish muscle proteins during *post mortem* storage at different temperatures. However, further investigations are needed in order to determine how these changes are related to the ultimate freshness of fish filet.

106T

Geometric morphometrics: a method for Rainbow trout stocks identification in aquaculture

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Stock identification is an interdisciplinary field that involves the recognition of self-sustaining components within populations and is a central theme in fisheries science and management. Characters used to identify fish stocks can be divided into three groups: those that are purely genetic, those that are purely environmental, and those that may reflect both genetic and environmental variation. Body shape is a difficult, but important, trait to quantify. Morphometric analysis provides a powerful complement to genetic and environmental stock identification approaches. We collected totally 2193 digitized images from the left side of 27 different stocks of Rainbow trout (*O. mykiss*), reared in 13 Trentino fisheries. Using TPS software package, 24 homologous landmarks were placed on each fish shape and rigor mortis arching effect was digitally corrected, then the landmark coordinates were adjusted using a generalized procrustes analysis (GPA). Finally we could emphasize the ontogenetic shape differences between samples. Afterwards the TPS output data (a points matrix) were analysed by NTSys statistical software, especially by a canonical variates analysis (CVA). CVA was preferred rather than principal component analysis (PCA) for simplifying descriptions between group differences. This method yielded evidence of morphological differences among different stocks, and within the same stock reared in different environmental condition. TPS software helped us to show these differences by a graphic method, in which the main variation axis is represented by the transition from a "lean" to a "fat" fish shape. In contrast, geometric morphometric method could be sensitive to artifices due to a wrong images acquisition procedure or rough sample displacement under the camera. More tests are necessary to proof the intraspecific discriminant power.

107T

Evaluation of exposure risk of *Sparus aurata* to AFB1: comparison of effects induced *in vitro* on SaHePs primary cultures and *Vibrio fischeri*

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Among all toxins produced by fungi of the genus *Aspergillus*, aflatoxin B1 (AFB1) is considered the most hepatotoxic metabolite often found in animal feeds and is involved as cause of decline in fish performances and health status as well as mortality. Nevertheless, the evaluation of the risk to AFB1 exposure in *Sparus aurata*, one of the most reared species in the Mediterranean, is still poorly investigated. Accordingly, the aim of this study was to compare the effects induced *in vitro* by AFB1 on a novel experimental animal model based on hepatocytes in primary culture of *S. aurata* (SaHePs) and on *Vibrio fischeri*, using a validate test system: Microtox[®]. In both *in vitro* systems, AFB1 exposure concentrations ranged from 1.60 pM to 32 µM with exposure times of 24, 48 and 72h in SaHePs and 5, 15, 30 min and 3.5h in *V. fischeri*. Cytotoxicity was assessed by measuring the retention of neutral red (NR) in SaHePs, while the light emission of *V. fischeri* was considered for Microtox[®]. In both assays results indicated that prolonged exposure times would cause a significant increase of AFB1 toxicity; equivalent and overlapping EC50/IC50 values were found at shorter exposure times as well as NOEC values (16 nM) at lower exposure doses. At values corresponding to LOEC (32 nM) in SaHePs, hormesis response was detected by Microtox[®]. At sublethal and subcytotoxic concentrations SaHePs cultures succeeded in characterizing the type of damage whereas Microtox[®] reported the hormesis range. Hence, SaHePs cultures could be considered a useful, innovative and species specific *in vitro* tool to evaluate the exposure risk to feedborne potentially dangerous substances like aflatoxins in aquacultured species.

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Monitoring of fish species in the Senio river: distribution and morphometric measures of the populations

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The aim of this work was to recognize and characterize the fish species in the Senio river, near Florence, in order to limit the loss of genetic biodiversity. These steps represent the basis for a subsequent valorisation of local species. Fish have been recognized as biological indicators for the protection of the quality of aquatic environments. In this study, monthly samplings were carried out during the year 2009 using the electric fishing on four different sites, two classified at *Ciprinidae* (C1 and C2 sites) and two at *Salmonidae* (S1 and S2 sites) according to the D.Lgs 152/2006. Data collected were used to estimate population density of each

identified species using the method of consecutive passages reported by Zippin (1958). Population density measures were used to calculate the semi-quantitative Abundance Index of Moyle (AI). Morphometric measures (weight, total length, standard length, minimum and maximum circumference) and the Condition Factor (CF, $\text{weight}/\text{total length}^3$) have been submitted to the analysis of variance considering the site as fixed effect (SAS statistical package). Barb, blageon, chub, south European nase and brown trout were collected. Chub was the most abundant species in every site considered (mean value AI 3.5). In the urban center of Palazzuolo (Firenze) city only the chub was collected, (AI 3 and CF 0.9). The most upstream site (S1) showed the greatest biodiversity (AI: Barb 3, blageon 2, chub 2, south European nase 4) presenting also a good population of brown trout (AI 2 and CF 0.9). The human impact appeared already in the S2 site localized upstream the city center in which the chub was confirmed more abundant (AI 4) than the other species which are more sensible to pollution. Nevertheless the Senio river resulted still suitable to guarantee life and development of fish populations even if the effect of human impact on water quality resulted important.

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An application of Z-Box method in dairy cow feeding to estimate the relationships among peNDF, other feed variables and productive dataMarcello Gozzi^{1,2}, Marco Cardelli², Flavio Melli²,
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Physically effective NDF (peNDF) is defined as the fraction of fibre that stimulates chewing and contributes to the floating mat of large particles in the rumen, and consequently to its regular activity. PeNDF is calculated from a physical effectiveness factor (pef), varying from 0 (NDF stimulates no chewing) to 1 (max chewing), which may be obtained by laboratory-based particle sizing techniques, such as Penn State Particle Separator, Mertens Separator, Z-Box, Cut Accuracy Test, based on the proportion of DM retained on sieves (by horizontal or vertical shaking). We chose Z-Box method, thanks to its easy use and applicability to as-is feed and total mixed rations (TMR), and we are trying to obtain an estimating equation which may predict milk fat content and/or other productive data from peNDF and other variables measured on TMR. To this aim, samples of TMR collected from several farms are sieved (3 sub samples each), and undergo proximate analysis, NDF, ADF, ADL and starch. Milk yield, milk fat, water addition to TMR are collected on farm; qualitative data such as type of forage, breed, season, geographical origin and altitude (plain/hill/mountain) are also taken into account, to estimate their possible effect. As a first step, in order to investigate the complex relationships existing among this wide set of variables, Principal Component Analysis (PCA) is used as a data exploration tool. Two PCA models (presence of silage or not in TMR) are calculated separately. For each PCA model, the overall correlations among all the considered variables and their relative importance are investigated by means of the loadings plots, posing particular attention to the correlations with peNDF and with milk fat. Moreover, it is also possible to identify how the different groups of samples depend on specific variables.

Acknowledgement

Thanks are due to PROGEO S.C.A. staff for the primary support to the research.

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Ruminal pH and redox potential as affected by devitalized and live yeasts (*Saccharomyces cerevisiae*) supplementation: an *in vitro* studyMassimo Trabalza Marinucci¹, Laura Mughetti¹, Claudio Forte¹,
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Many researchers have shown the ability of *Saccharomyces cerevisiae* to improve rumen efficiency and health by decreasing the production of lactate and enhancing fibre digestion by the cellulolytic bacteria. However, there is still debate concerning the mechanism of action of live yeasts (LY) vs. devitalized yeasts (DY). The objective of the experiment was to evaluate, using an *in vitro* fermentation system, the effects of either LY or DY on pH and redox potential (Eh). Ruminal fluid inoculum collected from sheep fitted with rumen cannulae was anaerobically incubated inside 4 jars with buffer solution (5:1 ratio) for 72 h at 39.5°C in a Daisy II Incubator (Ankom Tech., USA). Treatments were as follows: 1) control (CTR), i.e. buffered inoculum with ground alfalfa (3.6 g) and barley (3.0 g); 2) CYS, i.e. CTR with 1 g of cysteine monohydrochloride (used as positive control for its reducing activity); 3) DY, i.e. CTR with 0.40 g of Thepax[®] 100R (Dox-al, Italy); 4) LY, i.e. CTR with 0.24 g of Biosaf[®] (strain NCYC Sc 47, Lesaffre, France). Measurements of Eh and pH were made at time 0 and then at 3, 6, 9, 24, 30, 48, 55, 72 h after incubation. Four replications of the trial were performed. Data were analysed using an ANOVA model for repeated measures. The presence of yeasts slightly influenced the average pH inside the jar (CTR: 6.21; CYS: 6.21; DY: 6.23; LY: 6.24; P<0.001). Values of Eh (mV) were affected by treatment (CTR: -246.23; CYS: -288.96; DY: -261.83; LY: -261.81; P<0.001), being both LY and DY significantly lower than CTR and higher than CYS. No differences were observed between LY and DY. Further studies are required to assess the mechanism of action of DY and LY strains in both *in vivo* and *in vitro* trials.

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Agronomic traits and feed quality of different cultivars of yellow lupin (*Lupinus luteus* L.)Vincenzo Chiofalo¹, Vittorio Lo Presti¹, Massimiliano Pagliaro¹,
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Among the GM-free protein sources, lupin appears to be an interesting crop to satisfy dietary requirements of livestock and, at the same time, safeguarding the quality of final products. To evaluate the productive and nutritional characteristics of *L. luteus* cultivated in the Mediterranean environment, 3 sweet varieties, Dukat, Mister and Taper, were sowed on a volcanic soil in Eastern Sicily in plots of 6 m² three times replicated in a randomized block design. Agronomic, nutritional and antinutritional traits were evaluated. ANOVA of data was performed and, when appropriate, means were separated using Tukey's HSD test. The three varieties of lupin yielded 1.3 t · ha⁻¹ with 18.7 pod x plant, on average. Tables 1 and 2 report the results in the studied varieties of lupin. Data suggest *L. luteus* as a real promise for the livestock food chain also to reduce the marked deficit of GM-free high-protein feedstuff in the Mediterranean crop-livestock system.

Table 1. Chemical composition (g · 100 g⁻¹) on a DM basis (85 g · 100 g⁻¹, on average) and quinolizidine alkaloids content (mg · 100 g⁻¹).

<i>L. luteus</i>	CP	EE	NDF	ADF	ADL	Alkaloids
Dukat	34.30 ^{ab}	5.91 ^a	24.74 ^a	21.32 ^a	1.94 ^a	0.97
Mister	36.23 ^a	5.14 ^c	23.21 ^b	20.09 ^{ab}	1.49 ^b	0.90
Taper	32.20 ^b	5.57 ^b	21.7 ^c	19.43 ^b	2.38 ^a	1.36

Mean values with different letters in the same column are significantly different for P<0.05.

Table 2. Fatty acid profile percentage*, ratio and quality indices.

<i>L. luteus</i>	SFA	MUFA	n-3 PUFA	n-6 PUFA	n-3/n-6	AI [§]	TI [‡]
Dukat	16.88 ^a	26.86 ^b	7.97 ^b	48.29 ^b	0.17 ^b	0.06	0.11 ^a
Mister	16.20 ^a	29.50 ^a	6.74 ^c	47.56 ^b	0.14 ^c	0.06	0.10 ^{ab}
Taper	15.11 ^b	24.57 ^c	9.46 ^a	50.84 ^a	0.19 ^a	0.06	0.09 ^b

Mean values with different letters in the same column are significantly different for P<0.05.

*Concentration of each fatty acid was calculated as percentage of the sum of all the fatty acid methyl esters identified. [§]AI=Atherogenic Index; [‡]TI=Thrombogenic Index.

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Use of oral zinc at high concentration during the post-weaning period of piglets

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The aim was to assess the effect of high dietary zinc supplementation on growth and health parameters of weaned piglets. Ninety castrated male pigs of 28 days of age were assigned to three different groups of 30 each: Group Zn21, was fed with a diet containing 2500 ppm of zinc oxide for the first 21 days (D0-D21) and without zinc supplementation for the following 35 days (D22-D56); Zn56 was fed with a diet containing 2500 ppm of zinc oxide from D0 to D56; the control group (C) was fed without zinc supplementation

throughout the experiment. All the groups were fed on *ad libitum* basis. Each pig was individually weighed at D0, D21 and D56. Furthermore, daily amount of diets offered the groups was weighed and mortality rate was registered. Mortality was analyzed by using the Fisher's exact test and no difference was detected as one piglet died in Zn56 between D21 and D56, and two in Group C between D0 and D21. Data regarding weight of piglets and food were analysed by the GLM procedure. At D0 no statistical difference was registered among the weight of the groups (means were 8.85 kg for Group Zn21, 8.84 for Zn56 and 8.86 for C) and this indicated that the weight at the beginning of the experiment did not influence the successive results. Statistical differences among the groups were registered both at D21 (16.29 kg for Zn21, 15.50 for Zn56 and 17.22 for C; P < 0.05) and at D56 (43.09 kg for Zn21, 38.62 for Zn56 and 43.84 for C; P<0.01). On the whole piglets of groups C and Zn21 showed the best growth performances. Also the weight of food offered the groups was higher at P<0.001 for Zn21 and C (1.02 and 1.08 kg/piglet/day, respectively) than for Zn56 (0.93 kg/piglet/day). In our study, diets with high concentration of zinc oxide did not significantly improve neither the sanitary status nor the growth performances of weaned piglets.

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Carbon and nutrient release in soil amended with biochar from pig manure

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The disposal of animal manure from livestock farms is of growing concern from an environmental as well as an human-health point of view. The pyrolysis of solid animal waste for biochar (BC) production could constitute a solution to the problem. Biochar is the term used to indicate charcoal when it is produced specifically for agroenvironmental purposes. With the general goal of evaluating the potential of BC from animal waste as crop fertilizer, in this preliminary work we studied in a laboratory experiment the carbon and nutrient dynamics in a silty clay soil amended with BC from pig manure. Wood chip, currently used as raw source for BC production, was chosen as reference material. Treatments were: soil amended with pig manure (LC); soil amended with pyrolyzed pig manure (LT); soil amended with wood chip (CC); soil amended with pyrolyzed wood chip (CT); soil with no amendment (control, C). Biochar was obtained by treating at 420°C, in anoxic conditions, wood chip or pig manure with moisture content ≤10%. The CO₂-C release, and change in available P and inorganic N contents between the start of the experiment and after 4 wks were measured in the amended and control soils. The CO₂ levels significantly increased in the CC and LC treatments during the incubation

time, whereas they stayed close to the control values in soils of the CT and LT treatments. The P concentration decreased during the incubation time in all treatments. At the end of the incubation period the inorganic N availability was higher in the soil samples amended with BC than in those amended with untreated organic matter, even if not as high as in the control samples. On the basis of these preliminary results it seems that the incorporation of BC from pig manure or wood chip to soil may permit carbon sequestration for longer periods than those associated to the incorporation of untreated organic matter. The application of BC from pig manure enriches the soil with higher nutrient amounts than those supplied with BC from wood chip.

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Analysis of landscape metrics in the dairy systems of the autonomous province of Trento

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Discontinuous patterns of landholding, i.e. farms composed of a scattering of patches with different land use, are commons in the agricultural sector of Alpine regions. This fragmentation increases production costs, particularly for highly mechanized forms of agriculture, and indirectly leads to impacts in landscape and biodiversity value. This study aimed at highlighting differences in patch and land use patterns for intensive and extensive dairy cattle systems identified in the autonomous province of Trento. The study area (Trento province, north-eastern Italy) covers a total of about 6200 km² and is composed by 223 municipalities (average size 30 km²). A total of 595 dairy farms registered to the Breeders Federation of Trento Province were surveyed and classified (intensive *vs* extensive) on the basis of structural and management variables recorded by a direct visit. The parcels managed by each farm were extracted from the georeferenced cadastral map database (ArcGIS 10). Land use composition, patch-based metrics (total and per class number of patches, Shannon diversity index) and topographic features (slope and aspect obtained from a 5 m DEM) were assessed for the identified systems. A total of about 217 km² covering 128,000 parcels (on a total of 1352 km² of surface managed by agricultural farms) were managed by dairy farms; the average number of parcels was 202 ±183. Land use, patch metrics and topographic feature analysis evidenced a higher degree of complexity and fragmentation for extensive dairy systems. The latter manage mainly meadows and pastures (0.53 ha/livestock unit), also in the steepest areas difficult to mechanize. Arable crops are used only by intensive systems (0.12 ha/livestock unit), and are almost null in the traditional extensive farms. Further research will be addressed to evaluate the rule of dairy cattle systems in landscape and biodiversity maintenance.

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Weaning performance of Italian Simmental female calves

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The objectives were (i) to compare the weaning performance of Simmental (S) and Holstein Friesian (H) female calves and (ii) to test a weaning scheme based on a standard milk replacer (MR) and whole S milk (SM). In a first trial two groups of S and H females (8 calves per group) were weaned with the same feeding programme based on milk replacer (150 g/L, 23% crude protein, 19% crude fat) and a concentrate mixture (compound feed: chopped alfalfa hay, 9:1). In a second trial, 20 S female calves were divided into two groups and weaned with the same feeding programme based on MR of the first trial or on SM. Both trials lasted 12 weeks and animals were reared in individual boxes, weighted at 3 weeks intervals with the milk fed till the 9 week of age. Blood samples were collected within 3 d after birth and analysed for haemoglobin (Hb) content. Data were analysed per trial, according to a one factor design $y = \mu + \alpha_i + \varepsilon_{ij}$ (μ = overall mean; α = breed and milk type effect for trial 1 and 2, respectively). In the first trial both groups received a total of 41 kg of milk powder and consumed the same concentrate mixture (102 kg). The S calves had a higher initial live weight and Hb contents than H (45 *vs* 39 Kg and 7.3 *vs* 6.1 mmol/L, $P < 0.05$). The average daily gains in the whole trial were similar between breeds (740 and 717 g/d for H and S, respectively). In the second trial, the two groups had comparable initial live weights (42.5 ± 3.3 Kg) and Hb contents (7.8 ± 1.4 mmol/L). The two groups received the same amounts of milk (271 L), with similar protein contents (3.6-3.5%) and higher fat contents for the SM than the MR group (4.3 *vs* 2.9%). The concentrate mixture intake and the daily gains during the whole trial were higher for the SM than the MR calves (110 *vs* 97 Kg and 810 *vs* 740 g/d, $P < 0.05$). In conclusion the S and H females have the same performances in standard weaning programmes and the use of whole Simmental bovine milk allows to increase daily gains of S female calves.

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Enzymatic starch hydrolysis potential of raw and processed cereals

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Starch is the major carbohydrate source in diet for pigs and poultry and its structure has an impact on the rate of starch hydrolysis. Our objectives were to assess *in vitro* starch hydrolysis potential of several starch-rich materials and to investigate relationships between hydrolysis potential and chemical variables. For this purpose, 40 samples of cereal feeds (both raw and heat processed, HP) commonly utilized in animal nutrition were evaluated using an *in vitro* setup based on the procedure proposed by Xiong *et al.* (1990) that involved an amyloglucosidase incubation of the starch-rich materials. For each sample, an enzymatic starch hydrolysis potential coefficient (ESHP, g/100 g starch) was then calculated. Data were analyzed by the GLM procedure of SAS, significances were declared at $P < 0.05$ and the CORR procedure was used to evaluate correlations between ESHP and parameters of interest. ESHP showed large differences among cereal feeds ($P < 0.05$), ranging from 16.2 to 98.4 g/100 g of starch for sorghum and HP rice, respectively. In general, higher ESHP values were found for HP cereals when compared both to raw materials and to respective raw counterparts ($P < 0.05$). Negative correlations with ESHP were recorded only for prolamin (g/100 g CP) and for amylose (g/100 g starch) contents ($r = -0.69$ and $r = -0.67$, respectively; $P < 0.001$). This work showed that starch hydrolysis potential is extremely heterogeneous among cereal feeds. The described *in vitro* procedure could be used as a good ranking method for starch-rich materials, even if it seems to be more appropriate in order to discern unprocessed from heat processed materials rather than differentiate among raw cereals.

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Effect of the stage of maturity on enzymatic corn starch hydrolysis potential

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The aim of the experiment reported was to investigate the effect of kernel maturation on corn chemical composition and on enzymatic starch hydrolysis potential. For this purpose, grain samples from three corn hybrids were collected at $\frac{1}{2}$ milk line ($\frac{1}{2}$ ML), $\frac{3}{4}$ milk line ($\frac{3}{4}$ ML) and black layer (BL) stages of maturity. By using an *in vitro* multi-enzymatic procedure, a enzymatic starch hydrolysis potential (ESHP, g/100g starch), an hydrolysis index (HI) and the rate of starch digestion (Kd, hour⁻¹) were calculated. Data were analyzed by the GLM procedure of SAS and significances were declared at $P < 0.05$. Stage of grain maturity influenced both several chemical parameters, the ESHP, the HI and the Kd values ($P < 0.05$). The kernels showed a regular increase in DM, vitreousness, starch, crude protein and zein contents and a decrease in crude lipid content with advanced maturity ($P < 0.05$). Moreover

the ESHP values showed large differences considering the different stages of maturity ($P < 0.05$). Overall, at the $\frac{1}{2}$ ML kernels had the highest ESHP values at all time intervals ($P < 0.05$), whereas HI markedly decreased with advancing maturity (55.8 *vs* 49.6 *vs* 40.9 for $\frac{1}{2}$ ML, $\frac{3}{4}$ ML and BL stage of maturity, respectively; $P < 0.05$). The lowest Kd value was recorded for the $\frac{3}{4}$ ML stage of maturity (0.139; $P < 0.05$). Data reported in this work showed a marked decline in the rate of enzymatic starch hydrolysis during kernel maturation. The correct choice of maturity at harvest could be considered, along with growing and storage conditions, in an accurate corn management.

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Analytical and methodological factors affecting the determination of NDF ruminal degradation rate

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The dry matter intake (DMI) of dairy cows is influenced both by NDF and NDF digestibility (NDFD, % of NDF) of diet. In particular for forage digestibility, a +1 unit of NDFD of diet results in a +0.16 kg of DMI/cow/d. However, NDFD is a high variable parameter, ranging from less than 40% for lignified legume forages to greater than 90% for unligified forages and fibrous by-products. Other than NDFD value, also the rate of NDF digestion (Kd) should be known and imputed in nutritional models to obtain a correct energy evaluation of forages that are being used. A first-order mathematical approach has been proposed by Van Amburgh *et al.* (2003) to calculate Kd. The inputs required for kd calculation are NDF, lignin and NDFD measured from 6 to 36 hours of rumen incubations. These items are needed to calculate: available NDF substrate at time 0 and at each incubation time (A0 and An, respectively) and indigestible NDF (iNDF, calculated as lignin x 2.4). The 6 h NDFD value could be used to calculate Lag phase (TL). Earliest time were not considered usefully. The first-order model was used to calculate kd of 9 forages (i.e., 1 straw, 3 corn silages, 3 alfalfa and 2 regrass hays). Different conditions (fixed *vs* variable TL; single *vs* multiple time point calculations; measured *vs* calculated iNDF) were tested to obtain kd of forages. All tested conditions influenced ($P < 0.01$; MSE 1.300) Kd determination. In particular, Kd values of 3.29 and 2.92 (CV=25%) could be calculated using a fixed or variable TL, respectively. A greater variability (CV=32%) was reported when Kd was obtained by using measured *vs* calculated iNDF (2.71 *vs* 3.51, respectively). A high Kd variability (CV=31%) was also obtained using the single or multiple time point calculations (Kd values from 2.42 to 3.73 for 48h and 6&24h time point calculations, respectively).

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Effects of dietary protein level on milk production and efficiency of N utilization in dairy cowsGiacomo Panella¹, Francesca Petrerà^{1,2}, Maria Grazia Maianti³, Luigi Calamari³¹Azienda Sperimentale "Vittorio Tadini", Podenzano, Italy²CRA, Centro di Ricerca per le Produzioni Foraggere e Lattiero-Casearie, Cremona, Italy³Istituto di Zootecnica, Università Cattolica del Sacro Cuore, Piacenza, ItalyCorresponding author: luigi.calamari@unicatt.it

The objective of this experiment was to evaluate the effect of the reduction of dietary crude protein (CP), to strictly cover the metabolizable protein requirement, on milk production and efficiency of N utilization. The study involved 28 mid-lactating Italian Friesian dairy cows and was conducted as a cross-over design comprising two periods of 28 d each. The cows were assigned (14 per treatment) to one of 2 isoenergetic dietary treatments (TMR based on corn silage): basal diet (B) and Low Protein diet (LP) containing 15.5 and 14.2% of CP (DM basis), respectively. Average daily DMI and N intake of each treatment was calculated on a weekly basis. Individual milk yield was recorded daily, and milk samples were weekly collected and analyzed. Average daily secretion of N in milk (g/d) was calculated on a weekly basis. Equation based on urea clearance rate was used to estimate urinary N excretion on a weekly basis. Blood samples were collected at the end of each period and analyzed. Data were processed using mixed procedure for repeated measures. Neither milk yield nor milk protein content were negatively affected by the dietary CP. The urea content in milk and blood (22.4 vs 28.5 and 23.8 vs 30.7 mg/100 mL, in LP and B respectively; $P < 0.001$) was affected by the dietary treatment. The estimated N urinary excretion (both as $g\ d^{-1}$ and as percentage of N intake) was lower in LP than in B; conversely, the overall average efficiency of N utilization was greater in LP (33.8 vs 31.1% of N output with milk in percentage of N intake in LP and B respectively; $P = 0.05$). In conclusion results highlighted that a dietary CP lower than the practice standard feeding does not reduce milk yield of mid-lactating cows, improves the efficiency of N utilization, and reduces N urinary excretion.

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Chestnut tannin inclusion in dairy cow diets

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The soluble component of the feed protein (solCP on a CP basis)

represents a valuable source of nitrogen for the microbial protein synthesis in the rumen. However, in condition of inadequate energy availability or high rumen protein degradation rate, ammoniogenesis in the rumen might result with lower nitrogen efficiency. Because of their ability of forming insoluble complexes with protein fractions, tannins might increase the by-pass of protein, reduce ammonia yield in the rumen and urea excretion in milk. Six multiparous cows (43 ± 9 kg/d milk yield and 93 ± 31 d in milk) were randomly assigned to 3 diet groups in a replicated Latin square design. Treatment diets (TRT) were CTR+ (14.9% CP on a DM basis, 28.2% solCP), CTR- (14% CP/DM, 31% solCP) and the T in which the CTR- diet was added 80 g/head/d of a chestnut hydrolyzable tannin. Each experimental period lasted 3 weeks. Cows were kept in a common pen, individually fed by means of a Calan Broadbent[®] feeding system and individual milk yield was recorded at each milking. Data measured over time were subjected to ANOVA using the repeated statement in the mixed procedure of SAS. The experimental unit was the animal treated alike. The statistical model included the fixed effect of TRT, time of measurement and (TRT \times time of measurement) interaction. The random variable was the animal within the square. The T group had a higher ($P < 0.05$) DM intake (26.9 vs 25.6 kg/head/d) and lower ($P < 0.05$) milk urea content (21.8 vs 23.8 mg/100ml) compared to the CTR+. No differences among groups were observed on milk (38.9, 37.5, 37.6 kg/head/d, geometric mean), fat (1.49, 1.42, 1.47 kg/head/d, geometric mean) and protein yield (1.33, 1.31, 1.31 kg/head/d, geometric mean), respectively for CTR+, CTR- and T groups. When adding chestnut tannin to high solCP diets, the urea excretion could be reduced as a result of a probable different protein degradation rate in the rumen.

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Presence of trypsin inhibitors in conventional and low antinutritional factors content soya-beans

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Since the ban of the meat and bone meal the legume seed do not have alternative as protein source in animal feeding. Because of the high protein value, the soya-bean is widely used in monogastric feeding. However, the presence of trypsin inhibitors in raw seeds requires a heat treatment step to get rid of antinutritional factors which could reduce the growing efficiency of the animals. The possibility of using the raw soy bean in animal feeding could be of interest since the fat component of the seed equals energy available to the animal and it would represent a way of using home grown feed without additional cost for feed processing. Eleven variety of soya-beans (Aires, Ascarubi, Atlantic, Bahia, Cresir, Dekabig, Hilario, Nikko, PR92B63, Pedro and Sapporo) were collected in four different locations (Emilia, Friuli, Piemonte,

Veneto) and in 3 consecutive years (2006-2008) for a total of 132 samples, then analyzed for the presence of trypsin inhibitors according to the method of Kakade *et al.* (1974). Results were expressed as mg of trypsin inhibited per gram of analyzed sample. Data were normally distributed and subjected to ANOVA in the mixed procedure of SAS. The statistical model included the fixed effect of variety, place of sampling and variety x place of sampling interaction. The random variable was the year of sampling. The observed trypsin inhibited values (mg/g sample) ranged from 20.6 (Aires) to 52 (Nikko). The trypsin inhibited values of Aires, Hilario, Bahia and Pedro ranged from 20.6 to 23.8 (22.6 as average), whereas the Ascasubi, PR92B63 and Sapporo variety ranged from 27 to 39.8 (32.7 as average) and Dekabig, Atlantic, Cresir and Nikko ranged from 44 to 52 (47.8 as average). Average among groups were significantly different for $P < 0.05$.

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Effect of some phytoextracts on *in vitro* rumen digestion

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Plant secondary compounds, generally considered in the past as antinutritional factors, can be worth to be tested in animal production systems as alternative to traditional chemical allopathic drugs and to enhance animal health and performance but, in ruminants, attention should be paid for possible interference with rumen metabolism. Particular interest assume phytoextracts with claimed antioxidant, antiinflammatory or immunomodulant potential. Five plant extracts from *Curcuma longa*, *Andrographis paniculata*, *Ginkgo biloba*, *Salix chilensis* and *Echinacea angustifolia* were tested *in vitro* for their possible effect on rumen fermentations and feeds digestion. The trials were carried out by the Ankom Daisy^{II} incubator, testing three concentrations of each extract (20, 60 and 120 mg/L). Corn meal, grass hay, alfalfa hay and beet pulp were incubated in F57 bags. Dry matter digestibility, pH, ammonia and VFA concentration were measured after 48 h of incubation. *S. chilensis* slightly reduced pH ($P < 0.05$). *G. biloba* and *S. chilensis* did not affect feeds dry matter digestibility, that was increased by the addition of *A. paniculata* in fibrous feeds in a dose dependent manner, but not in corn meal. On the contrary, *C. longa* extract slightly but significantly reduced digestibility of all feeds samples and also ammonia concentration. All plant extracts did not significantly alter VFA concentration, suggesting a possible effect on microbial efficiency: ameliorated by *A. paniculata* and lowered by *C. longa*.

All the tested plant extracts did not induced major changes in rumen metabolism, at least when evaluated *in vitro*, and can be candidates for *in vivo* utilisation. Nevertheless, their *in vivo* effects should be tested, mainly for long lasting utilisation and with attention to their possible consequences on whole digestive tract.

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Feed palatability affects feeding behaviour of calves

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The aim of this study was to assess the impact of feed palatability on feed intake and feeding behaviour of growing calves. Ninety Holstein calves were blocked by initial BW (158 ± 20.5 kg), distributed into 6 pens (15 calves/pen), and allotted to 2 treatments (3 pens/treatment) for 26 days (7 d of adaptation and 19 d of study). Animals were fed *ad libitum* ryegrass hay and a complete pelleted feed (CP 14.2%; NDF 18.2%) either plain [Control (C)] or sweetened [Sweet (S)] with 500 g/ton of a high-intensity sweetener (Luctarom[®] SFS-R, Lucta SA, Spain). Each pen was equipped with individual mangers connected to an automatic recording system that allow monitoring the number of meals, meal duration and the amount of feed consumed during each meal. Data were analyzed using a repeated measures structure with a mixed-effects model that included animal as random effect, and treatment, time, and their interaction as fixed effects. Although the time animals spent eating was similar between treatments, there was a significant interaction between treatment and time ($P < 0.01$) for the number of daily meals. This happened because in 8 of the 19 experimental days, calves fed S took on average 6% more meals ($P < 0.05$) than those fed C (9.7 vs 10.3 meal/d). In addition, feeding S increased ($P < 0.01$) intake rate compared to C (20.2 vs 18.9 g/min, respectively). Although differences were not significant, calves fed S consumed 5.8% more concentrate than their counterparts (4.9 vs 5.2 kg/day). In summary, improving diet palatability increased intake rate and the number of daily meals, albeit the later effect was not consistently observed throughout the experiment. Results suggest that feed palatability may create opportunities for managing the feeding behaviour of calves.

124F
Prediction of net energy of lactation content of corn silage and alfalfa hay

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Aim of the trial was to predict the net energy of lactation (NEL) of corn silage and alfalfa hay grown in the Po Valley. Five Italian university laboratories collected 120 samples of each forage during the autumn 2008 and 2009 in 16 provinces of five Italian regions (Piemonte, Lombardia, Emilia Romagna, Veneto and Friuli Venezia Giulia). Chemical contents (DM, ash, ether extract, crude protein, NDF, ADF, NDIN and ADIN) and NDF digestibility (NDFD) were determined according to NRC (2001) to calculate the NEL adjusted for a level of intake at 3×maintenance (NEL3x, NRC 2001). The NDFD was measured *in vitro* by using filter bags, inserted into rotating digestion jars of a Ankom DaisyII fermenter for 48 h. Chemical composition of alfalfa showed greater variability coefficients (from 14 to 20%) than corn silage (from 9 to 14%), and lower and more variable NDFD values (36.2±6.6 vs 49.2±6.6%). The average NEL3x of corn silage (1120±122 kcal/kg DM) and alfalfa (1484±84 kcal/kg DM) were similar to average tabulated values of INRA (2007) and NRC (2001). The following multiple regression equations, based on ash, NDF (% DM) and NDFD (%NDF), were obtained to predict the NEL3x contents of forages:

$$\text{Alfalfa hay : NEL3x (kcal/kg DM) = (100-ash)/100 * [1499 - (16*NDF) + (13*NDFD)] R}^2 : 0.93.$$

$$\text{Corn silage: NEL3x (kcal/kg DM) = (100-ash)/100 * [1603 - (15*NDF) + (11*NDFD)] R}^2 : 0.96$$

Both equations allow a simpler prediction of NEL3x than those based on a complete set of chemical assays as required by NRC (2001).

125F
The effect of homefermentative bacteria and enzyme on the fibre characteristic of corn silage in different time point

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Improving the nutritive value of corn silage for dairy cattle is an important goal. In Southern Italy the agronomic and environmental conditions are not so good to guarantee an optimal corn silage quality. The objective of this study was to determine how an inoculant containing homofermentative bacteria plus enzyme (*L. Buchneri*, *P. Pentosaceus* *Beta-glucanase* *Xylanase*) would affect fibre digestibility and aerobic stability of corn silages. A field experiment was conducted in a farm located in Southern Italy, Molise region, during the growing season of 2009. For this study silage corn hybrids Pioneer 36-B-08 were used. The corn was harvested at the end of September. Chopped forage was divided into two 30-kg piles. One pile was treated with 200 mL of deionized water (C), and the remaining pile was treated with the inoculant containing solved in 200 mL of deionized water (T). Triplicate corn samples of 1.5 kg each were vacuum sealed into polyethylene bags and stored for 30, 60, 120 and 180 die in the laboratory (21°C) in black plastic bags during all the experimental time. For each time and for each thesis corn silage was analyzed for chemical composition, pH, N-NH₃, buffer capacity and NDF digestibility at 24-48 hours. The chemical composition of freshly chopped forage was not different between treatments. The concentrations of CP and fibre fractions (NDF-ADF-ADL) after fermentation were not affected by inoculation at all time point. The pH and ammonia-N of the silages were similar between treatments at all sampling times, with the exception of pH that was higher in inoculated silages at 180 d (P<0.05). The buffer capacity was also higher in T silage than in C, in all experimental time (P<0.05). Inoculant did not affect the NDF digestibility, even if we observed a tendentially increasing in treated corn silage.

126F
Effects of different feeding systems of Apuan Alps goat population on growth rate and puberty age

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Only 50-60% of Apuan Alps goat population, kept on pasture, reaches puberty within the first year of life. To increase this percentage a feeding trial was carried out. Twelve goats were divided into two groups homogeneous for birth weight (P: 2.38±0.12 kg; S: 2.38±0.11 kg). Goats of group P were fed mother's milk and supplemented with alfalfa hay and maize grain until the age of 90 days. Then they were kept on pasture at 500-1700m a.s.l. in Apuan Alps park. Goats of group S were kept indoor and fed replaced milk and supplemented with alfalfa hay and mixed feed (16.5% CP) until the age of 90 days. Then they were fed alfalfa hay and the

same mixed feed. All animals were weighed every 15 days until the end of the trial at 8 months of age. The data analysis showed a significant ($P=0.017$; $SEM=1.46$) final live weight differences between animals of group P (26.2 kg) and group S (32.2 kg). Average daily gain of group S (113 g/d) was significant higher ($P<0.0001$; $SEM=7.1$) than that of group P (97 g/d). Puberty age was reached only by 4 animals of group P (287 ± 6.4 d) and by all animals of group S (246 ± 9.4 d). All these animals were pregnant and gave birth except a subject of group P that aborted late. The trial showed a positive effect of intensive feeding on the number of animals reaching puberty during the first year of age.

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Effect of different feeding systems on intake and growth of young bulls of Sarda breed

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A study was carried out (May-July 2010) at the Foresta Burgos experimental farm, to evaluate the effect of different feeding systems on nutrient intake and growth of Sarda young bulls. 28 Sarda young bulls were divided into 4 groups (7 heads each) homogeneous for live weight (LW, 288.7 ± 5.5 kg), BCS (2.69 ± 0.03) and age (355 ± 5 days). One group grazed 24 h 7-ha natural non irrigated pasture (group PAS) and three groups were kept in barns and fed daily with natural pasture hay (ad libitum) and concentrate: 2.5 kg/head (FC1), 3.3 kg/head (FC2) and ad libitum (FC3). Herbage mass on offer was measured by clipping at ground level fifty 0.5 m². The chemical composition of the feedstuff used was measured. The individual herbage dry matter intake (DMI) and *in vivo* DM digestibility (DMD) were assessed (n-alkane method) in the group PAS. The DMI and DMD (acid insoluble ash marker method) of hay-concentrate diets, as group average, were detected in groups FC1, FC2, FC3. Every three weeks body weight and BCS were recorded. All data were tested by GLM using the diet as fixed effect. The herbage mass on offer was 4.7 t DM/ha. The energy (EN, Mca/kg DM) and CP concentrations in the four diets were 1.16 and 12.6%, 1.33 and 12.6%, 1.42 and 13.4%, 1.72 and 16.7% respectively in PAS, FC1, FC2 and FC3. DMI in the pasture-based diet was 8.6 kg/head, with DMD of 64.4% whereas in the hay-concentrate-based diets DMI and DMD were: 6.2 kg/head and 59.3% in FC1, 6.9 kg/head and 65.8% in FC2, 10.1 kg/head and 56.2% in FC3. As expected, the FC3 group showed the highest ($P<0.001$) growth rate (1.21 ± 0.06 Kg/d) and BCS (3.01 ± 0.05) followed by PAS (0.78 ± 0.06 kg/d and 2.67 ± 0.05), FC2 (0.75 ± 0.06 kg/d and 2.57 ± 0.05) and FC1 (0.58 ± 0.06 kg/d and 2.45 ± 0.05). Diet based on pasture for young bulls of Sarda breed seems to be a valid chance for Sardinian cattle livestock system.

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Effect of methionine hydroxy analog isopropyl ester on performance of early and mid lactating dairy cows

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Aim of this trial was to evaluate the hypothesis that a low crude protein (CP) diet supplemented with the isopropyl ester of the 2-hydroxy-4(methylthio) butanoic acid (HMBi-Metasmart™) would increase dairy cows performance without negative effect on N excretion. Thirty-two Italian Friesian cows of which 16 early lactating (120 ± 60 DIM) and 16 mid lactating (215 ± 70 DIM) were used to compare two diets (22g/day HMBi and no Met- supplementation) with the same CP level (14,5% on DM). HMBi is used to optimise Lys:Met ratio that were respectively 3:1 and 3,4: 1. The experimental design was a change over within stage of lactation, with four groups and two periods lasting four weeks (2 week adaptation + 2 week sampling). According to treatment and stage of lactation dairy cows were divided into 4 groups: Early control (CE) without HMBi; Early Metasmart™ (ME) with HMBi; Mid control (CM) without HMBi; Mid Metasmart™ (MM) with HMBi. N excretion was estimated by milk urea content, milk yield and protein content. Data were analysed with ANOVA using the MIXED procedure of SAS, with treatment, group and their interaction as main effect, and cow within group as random effect. Available data showed that milk and protein yield were significantly higher in MM than in CM ($P<0.01$) while were not affected by HMBi supplementation in early lactating cows (CE and ME). Observed data showed also that there was no difference between stage of lactation and between feeding treatments, but high performance dairy cows (CE-ME) thanks to higher N intake, produced milk with a higher N content in comparison with low performance dairy cows (CM-MM). Referred to dairy cows aminoacids requirements, HMBi diet fulfil them optimising Lys:Met ratio in MM that reached higher yield levels than that fed without supplementation (CM). On the other hand early lactating dairy cows available data showed that 22 g/d of HMBi were not probably enough to see an increase in dairy cows performances.

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Effect of lycopene supplementation on cryosurvival of turkey spermatozoa

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Sperm health is a key factor in male fertility and lycopene, as an antioxidant, may help maintain semen quality, but its efficacy in avian spermatozoa has been poorly studied, especially during sperm cryostorage conditions. This study aimed at evaluating the effects of lycopene supplementation in freezing extender on turkey sperm quality following cryopreservation. Five pools of turkey semen were each divided into 3 aliquots that were diluted with the Tselutin extender containing 0 (control), 0.05, or 0.1 mg/mL lycopene, respectively. Semen was frozen by adding 80 μ L of seminal aliquots directly into liquid nitrogen to form frozen pellets that were subsequently thawed at 75°C in few seconds. Sperm mobility (Accudenz swim-down test), viability (SyBr-PI staining), osmotic-resistance (HOS-test) and DNA integrity (Acridine orange staining), were determined on both fresh and frozen/thawed turkey semen. Results showed that mobility, viability and osmotic-resistance of turkey spermatozoa were impaired by cryopreservation ($P < 0.05$), independently by the addition of lycopene in freezing medium. Conversely, DNA integrity was affected by cryopreservation only in the control ($P < 0.05$), in fact in lycopene-supplemented samples the percentages of DNA intact sperm did not differ from those of fresh semen. In particular sperm DNA integrity recovered post-thaw was significantly higher ($P < 0.05$) in sperm cells exposed to a concentration of 0.1 mg/mL of lycopene than in cells frozen without lycopene (90.05% vs 95.06%). These data suggest that a lycopene-rich extender could offer protection against the DNA damage that occurs in turkey spermatozoa during cryopreservation.

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Accuracy of two *in vivo* methods to assess body condition of rabbit doesCesare Castellini, Alessandro Dal Bosco, Raffaella Cardinali
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Body Condition Score (BCS) and Perirenal Fat Thickness (PFT), through ultrasound scanner of 40 multiparous does with different sized litters were assessed. Ten does were slaughtered 1 day after parturition, the others nursed 5 (group L5; $n=15$) or 10 kits (group L10; $n=15$). At weaning (day 30 of lactation) all does were sacrificed, adipose tissue excised and weighted. The does were weighed daily and subjected to ultrasound scanning and BCS eval-

uation. On the day of kindling does were subdivided into two classes on the base of their body condition: does with high (BCS > 3 and PFT > 0.72 cm) and those with poor body condition (BCS < 2 AND PFT < 0.72 cm). The BCS evaluation was based on the test of the loin and rump region for bone protrusions and muscle fullness. An aggregate BCS of the loin and rump regions was calculated (0-4). The effect of litter size were analyzed with a linear model (STATA 2005). At weaning the L10 females showed a worse body condition and a 35.8% lower weight of adipose tissues ($P < 0.01$). Analyzing the BCS/PFT effect, L10 group in both group, showed a strong reduction of final BCS ($P < 0.05$), although the feed intake is the same. The calibration curve obtained were accurate enough and give a good indication of PFT ($R^2=85.55$; RSE 6.11). The present research showed that perirenal fat thickness and body condition largely decrease when rabbit does nurse large litters. Ultrasound measurement of perirenal fat thickness may be an accurate method for *in vivo* monitoring the fat deposit; BCS is a less precise measure for perirenal.

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Use of transabdominal ultrasonography to characterize the ovarian status in rabbits

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Advancements in imaging technologies over the last two decades have ushered a quiet revolution in research approaches to study reproductive biology and pathology of many domestic animals. Serial *in vivo* examination can permit detailed evaluation of ovarian structure and function and led to a deeper understanding of endogenous mechanisms controlling follicular and luteal dynamics also in rabbits.

Abdominal skin is shaved and covered with scanning gel. A 12-mHz linear-array probe with a real-time B-mode ultrasound scanner (SSD-900; Aloka Inc.®) are used and the non-sedated rabbits lay in a dorso-lateral position. The setting of the scanner that affects image attributes (i.e. overall time-gain, near-field and far-field gains, compensation and beam focus) is kept at predetermined levels. Images are displayed at the maximum magnification. The ovaries are located by first identifying the kidney as a major landmark, and then moving the transducer a few millimetres caudo-laterally from the caudal edge of the kidney, remembering that the ovaries have a superficial location under the skin. The best technique is to start with the left ovary, which is in general easier to find. Individual follicles on each ovary are identified, measured and classified according to the following size criteria: small follicles (< 1.5 mm) and large follicles (> 1.5 mm). The follicles generally appear as anechoic wide circular areas. However, when numerous within the same ovary, they are in some case flattened and packed together and the risk to underestimate the total number should be considered. CL are visible as moderately hyper-

echoic structures. The occurrence of ovulation is evaluated by the presence of CL, since rabbits are induced ovulators and functional CL should not be present in the ovary of unmated females. The ovulation rate is determined by counting the number of CL on both ovaries. The use of ultrasonic imaging may improve rabbit industrial production because it allows to assess reproduction efficiency in a direct, noninvasive, and atraumatic manner without interruption or distortion of ovarian function.

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Effect of foot rest on sore hock of rabbit does

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The aim of the experiment was to examine of sore hock depending on the housing condition. Crossbred female rabbits (n=108) were housed into four different cages: Flat NO: flat deck cage without foot rest (n=30); Flat FR: flat deck cage with foot rest (n=30); Plat FR: cage with wire net platform, foot rest on the lower level (n=24); Plat PLAST: cage with plastic platform (n=24). The hind legs were examined at each insemination. The incidence of sore hock was scored according to Rommers and Meijerhof (1999): 0= foot pads intact; 1= no hairs, callus formed (<2.5cm); 2= no hairs, callus formed (>2.5cm); 3= callus open, craches have been found, 4= wounds. The incidence of sore hocks (intact or less serious: score 0, 1, 2; and more serious, which cause pain: score 3, 4) in different groups were statistically compared by Chi-square test using SPSS 10.0 software package. At the beginning of the experiment no sore hocks were observed. The percentage of rabbits with score of 0 1 and 2 were 100, 95, 100 and 52%, and that of score of 3 and 4 were 0, 5, 0 and 48% at the 5th insemination in groups Plat PLAST, Plat FR, Flat FR and Flat NO, resp. (P<0.01). With increasing the time spent in production the incidence of sore hock and their severity increased. In group of Plat PLAST there were no observations for sore hock of scores 3 and 4 but in group of Flat NO its ratio was 48% at the 5th insemination. A connection was found between the body weight of does and the incidence of sore hock. It can conclude that the incidence and severity of sore hock were the lowest in cages with plastic platform and highest in cages without foot rest. Inserting foot rest in cage of rabbit does can be recommended from the viewpoint of welfare.

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Group housing of rabbit does

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Some bio or organic rabbit production systems and specialists suggest group housing of rabbit does. The aim of the experiment was to compare the production of does housed individually (I) or in group (G). In group I 18 does were housed in commercial cages (size: 0.32 m², height: 0.3 m). In group G 4 does and 1 buck were housed to each of four pens with 7.7 m² (half of the floor was deep litter and the other half plastic slat) with 4 nest boxes in each. Similar reproductive rhythms were applied: rabbits were mated or inseminated after parturition. The kindling rate and suckling mortality were evaluated by means of Chi-square test, the survival of does was analyzed by Survival-analysis and the litter size by two-sample t-test, using SPSS 10.0 software package. During the five reproductive periods the percent of does kindled 0, 1, 2, 3, 4, 5-times were 0, 0, 23, 69 and 8%, and 17, 25, 17, 25 and 16%, the kindling rate was 77.6 and 45.6%, respectively in groups of I and G. No significant differences were observed for litter size. The suckling mortality was 14.0 and 38.5%, and the survival of does was 71 and 50%, respectively in groups of I and G. The corticosterone levels were 61 and 175 nmol/g. The high mortality of kits was partly connected with stress and aggressive behaviour of does: scratching, biting or killing the kits. The high stress caused the high killing rate and shorter life-span. Authors observed similar problems for European wild rabbits (the social rank order is close connected with corticosterone level, heart rate, number of litters and kits born per year, lifespan, infanticide). The benefit of wild rabbits living in groups is the reduction of predation risk but in case of domesticated rabbits only the cost of group living remain. The conclusion is that group housing of rabbit is detrimental for animal welfare.

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Influence of *Lactobacillus acidophilus* and *Lactobacillus plantarum* supplementation in drinking water on egg quality and layer performance

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Probiotics or direct-fed microbials are live microorganisms which have beneficial effect for the host through improvement of intestinal microflora. In particular, *Lactobacilli* are commonly used as probiotics in poultry production. The aim of this study was to evaluate the effect of a mixture of *L. acidophilus* and *L. plantarum* administered with drinking water on egg quality and layer performance. The trial, performed on two different farms, was conducted on two groups of 240 laying hens which were housed in pens and fed *ad libitum* the same commercial diet. From 21 to 44 weeks of age, in each farm animals were divided into two groups: the hens of the first group received water with the supplementation of 108 CFU/animal/day of the experimental mixture (Treatment = T), while animals of the other group received water without any supplementation (Control = C). Number and weight of eggs were recorded daily, while feed consumption and body weights of the animals were registered every week and monthly, respectively. At 25, 30, 35, 40 and 44 weeks of age, specific gravity of eggs, albumen quality (Haugh Units) and shell thickness were measured on 30 eggs/group. The supplementation of the blend did not affect body weight, daily feed intake and feed conversion efficiency of the animals during the experimental period. No significant difference was observed in egg production and egg mass of hens between the two groups. The effect of treatments on egg quality showed a higher specific gravity and Haugh Units of eggs in the T group in comparison to the C group. In conclusion, the supplementation of this mixture in drinking water did not influence growth performance of hens, but seemed to improve some characteristics of egg quality.

135F

Study of Italian hare (*Lepus corsicanus*) in different habitats of southern Apennines in Basilicata region. I. Floristic composition of diet

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In the Park of Gallipoli Cognato was carried out a study on diet composition of Italian hare. Faeces were monthly collected during 1 year in 3 different sites with the following botanical associations: site 1 (370 m a.s.l.), *Viburno-Quercetum ilicis*, *Roso semper-virenti-Populetum nigrae*, *Pruno-Rhamnetalia alaterni*; site 2 (532 m a.s.l.), *Pruno-Rhamnetalia alaterni*, *Thero-Brachypodietaea*; site 3 (810 m a.s.l.), *Centaureo-Quercetum pubescentis*. From faeces, by histological analysis, were identified 78 species. Their inci-

dence was determined by Brawn Blanquet method. The most representative species in diet were: *Allium subhirsutum*, *Prunus spinosa*, and *Brachipodium sylvaticum* in site 1; *Eryngium campestre*, *Brachipodium pinnatum*, and *Agrimonia eupatoria* in site 2; *Picris hieracioides*, *Allium subhirsutum*, and *Brachipodium sylvaticum* in site 3. Kruskal Wallis's test was performed on nonseasonal diet species shared by the three sites. Their incidence was tested between sites within period, and it was observed that: content of *Lolium rigidum* varied with seasons ($P < 0.001$) (Spring $\chi^2 = 12.65$, Summer $\chi^2 = 44.94$, Autumn $\chi^2 = 19.6$, Winter $\chi^2 = 20.97$); presence of *Allium subhirsutum* was significantly different in Spring ($\chi^2 = 14.49$, $P < 0.001$), Autumn ($\chi^2 = 29.05$, $P < 0.001$), and Winter ($\chi^2 = 6.00$, $P < 0.05$); *Trifolium pratense* varied in Spring ($\chi^2 = 5.95$, $P < 0.05$), and Autumn ($\chi^2 = 39.09$, $P < 0.001$); incidence of *Plantago lanceolata* in diet was significantly different ($P < 0.001$) in Spring ($\chi^2 = 18.23$) and in Winter ($\chi^2 = 2.17$); *Carex distachya* and *Poa trivialis* varied in Spring ($\chi^2 = 7.71$, $P < 0.05$) and in Winter ($\chi^2 = 22.35$, $P < 0.001$). This study evidenced that feeding of Italian hare is based principally on few species but, even if in low quantity, a great amount of plants typical of the different habitats accomplishes diet.

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Study of Italian hare (*Lepus corsicanus*) in different habitats of southern Apennines in Basilicata region. II. Vegetational aspects of trophic niches

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This study was carried out in the Regional Park of Gallipoli Cognato on three sites that differed for floristic and vegetational aspects, in order to characterize trophic niche of Italian hare. Faeces were sampled monthly from March 2009 to February 2010, bleached in hypochloride solution, washed, mixed, and mounted on microscope slides. Coverage of species observed in each slide was estimated by abundance-dominance index (phytosociological sampling method). Matrices obtained from each trophic site were submitted to cluster analysis. Data were treated by Multivariate analysis procedure (Syntax 2000-5.2 software). Moreover, a PCA diagram was performed on the same data set following an ordering according to PCoA. The multivariate analysis lets us to identify the clusters corresponding to the different phytocoenosis that occurs during the year, while in the PCA ordering species are assembled in groups for ecological affinity needs. In site 1

(matrix=60 species x 205 slides), most of species are perennial, sciaphilous and moderately mesoigrophilous and come from undergrowth and clearings of Mediterranean evergreen forest with few seasonal differences. In site 2 (matrix=60 species x 246 slides), resulted 3 clusters: a first group formed by heliophilous, emicriptophyte, and bulbous species with development mainly in Winter and in Spring; a second group composed primarily of annual herbs; a third late Summer and autumnal group in which there are many bushes, especially *Rosaceae* with fruit production. In site 3 (matrix=67 species x 252 slides), we highlight two groups: the first one made up of heliophilous species and coming from the pastures, the other one dominated by forest bushes and undergrowth herbaceous species.

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Brown hare (*Lepus europaeus*) in Basilicata region: multi-criterial evaluation for land suitability

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In this study was evaluated Brown hare habitat suitability in land

area of Basilicata region utilizing a multicriterial evaluation procedure (MCE-GIS model). Factors included in calculating the suitability model were: altitude, slope, exposition, annual mean temperature, annual mean precipitations, woodlands, areas of sparse vegetation, natural pasture and high altitude meadows, pollution, noise, and stray dogs. Each factor was evaluated and standardized by membership fuzzy and crisp functions. According to literature and expert knowledge threshold values were chosen in order to explain, for each factor, the change of suitability of area by Analytical Hierarchy Process (AHP). The eigenvector of weights (consistency ratio=0.04) put in evidence that the most important factors resulted: altitude ($w=0.23$), annual mean temperature ($w=0.22$), and precipitation ($w=0.17$). Factors were successively aggregated according to Weighted Linear Combination, (WLC), and after a last values discretisation by Chen method, territorial classification was effected. In such a way, regional territory was divided into 4 classes of suitability: 1, absent; 2, low; 3, mean; 4 high. This study evidenced the good hare suitability of most of the regional territories: on a total surface of 10.000 km², over 81% was composed with "mean" and "high" suitability lands. At provincial level the incidence (%) of classes resulted, from the lower to the higher class, respectively: 2.32, 5.53, 65.31, and 26.84 in the Province of Potenza; 17.52, 23.01, 39.54, and 19.94 in the Province of Matera. This methodology is going to become a needful tool in wildlife and in hunting management. For this reason it would be auspicious to make this method an even more effective instrument by considering, if necessary, new factors, and increasing the share of the methodological approach.

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Effect of grazing management on fat indoles concentration in lamb

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Literature shows that indole and skatole production in the rumen and their accumulation in fat tissues are strongly affected by herbage composition, which, on its turn, can be affected by pasture management. For this purpose, an experimental trial was conducted to observe the variations in lamb perirenal fat indoles concentration among different grazing management systems. Thirty-five Merinizzata Italiana male entire lambs (average body weight: 16.1 kg±SD 2.1 kg) were blocked in groups of 4. Over 72 days, animals were allowed to graze a 1 ha regrass (*Lolium perenne*) sward. Twelve lambs (continuous grazing, CG) grazed from 9 am to 5 pm, eleven lambs (morning grazing, MG) grazed from 9 am to 1 pm and twelve lambs (afternoon grazing, AG) grazed from 1 pm to 5 pm. Herbage was sampled at 3 different hours (both in MG and AG groups) and at 6 different hours (in CG group) in order to determine water soluble carbohydrates (WSC) content. Samples of perirenal fat were collected from each carcass and then analysed by GC-MS for indole and skatole determination. Average herbage WSC content was affected by the grazing systems. It resulted significantly higher ($P<0.05$) in herbage grazed by AG lambs compared to MG ones; herbage consumed by CG group showed intermediate values (106.1 vs 75.7 and 87.9 g/kg DM respectively for AG, MG and CG). MG group showed significant higher indole concentration in the perirenal fat compared to CG group ($P<0.05$) and higher value than AG group in tendency ($P<0.1$). No differences in indole were observed between CG and AG groups. Skatole was not statistically different among groups. These results seem to demonstrate that grazing management system affects perirenal fat indoles, probably associated with the different WSC availability.

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Data from preliminary investigations on acorn production in organic outdoor pig farming

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The aim of this study is to estimate the amount of nutrient from

woodlands in organic outdoor pig farms. Oak acorns are a high value food resource for outdoor pigs. Acorn production is influenced by several factors, especially a typical “year-on-year” variation. So every year it is necessary to calculate the amount of nutrients from acorns to evaluate the correct feed intake and balance the diet. According to official methods of sampling, acorns were collected by hanging several traps on oaks to define their number, weight and nutritional value. We applied this method to three different outdoor pig farms (A, B, C), with different species of oaks (*Q. cerris*, *Q. pubescens*). Farm A was at 900 m ASL. The examined paddock area was 18 ha, partially wooded mainly with oaks. The oaks were tillers and saplings of *Q. cerris*, only saplings were productive (average 6 m Ø foliage). Farm B was at 750 m a.s.l. The examined paddock area was 3 ha, partially wooded mainly with oaks (*Q. pubescens*) (average 9 m Ø foliage). Farm C was at 20 m ASL. The examined paddock area was 10 ha with some isolated oaks (*Q. pubescens*) (average 20 m Ø foliage) on grassland. The sampling was carried out during the acorn production period from September to December. We calculated the single tree acorn production, on farm A (13.61±2.27 kg DM), on farm B (6.63±1.03 kg DM) and on farm C (137.60±27.52 kg DM). The nutritive characteristics of acorns were on farm A 61% DM, 4.10% CP and 3.90% CF, on farm B 61% DM, 4.56% CP and 3.58% CF and on farm C 60% DM, 7.36% CP and 3.14% CF. Fatty acid composition of acorns was SFA% from 18.64 to 19.02, MUFA% from 43.26 to 53.92 and PUFA% from 25.44 to 34.68. Further investigations are necessary to determine the range of production of oaks and the relative changes needed through supplement pig diet in relation of an optimization of the nitrogen balance.

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Effect of two different packaging materials on physical and oxidative properties in veal calf fresh meat

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The aim of the work was to evaluate the effect of two different packaging materials on some oxidative and rheological parameters. For the trial were used 8 Fresian veal calves. Sixteen samples of *Longissimus dorsi* were taken from the right half of each veal calf, packed with two commercial films with different chemical and physical properties. Eight samples were packed with Cryovac® film (25 µm thick) and eight were packed with Weegal® film (45 µm thick). In both the packagings was used the same gas mixture (O₂ 46%, CO₂ 23%, N₂ 31%) and stored at 4±0.5°C until the analysis that were performed at the following post-packaging times: 0, 2, 4, 6, 8, 10, 12 and 14 days post-packaging. Chromatic parameters, drip loss, cooking loss, water holding capacity, protein oxidation, Tbars and hydroperoxides production were measured at each

time and in both the packaging thesis. Data were submitted to two-way ANOVA for repeated measures and to Tukey test, considering as independent variables the packaging material, the post-packaging time and their binary interaction. All the investigated parameters showed to be highly influenced by the packaging material and time ($P < 0.001$). Veal calf meat packed with lower gas permeability film (Weegal[®]) showed lower oxidation of fat and proteins ($P < 0.001$), lower chromatic indexes ($P < 0.001$) and an higher water holding capacity ($P < 0.001$). Moreover, oxidative parameters increased during storage ($P < 0.001$), instead water holding capacity decreased ($P < 0.001$). Meat oxidation influenced chromatic parameters. In fact, b^* values increased during storage time ($P < 0.001$) and a^* values increased until 4th day, due to myoglobin oxygenation, and decreased for its oxidation ($P < 0.01$). The material characterized by higher thickness, and by lower O_2 and CO_2 permeability, revealed to be the better film in veal calves fresh meat packaging because of its effect in reducing chromatic, chemical and physical degradation processes.

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Action of calpains and calpastatin on meat tenderness in diaphragm muscle of Marchigiana cattle breed

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The calpains are Ca^{2+} dependent proteases, divided in μ - and m-calpain based on amount of calcium required for their activation (5-50 μM for μ -calpain; 0.2-0.6mM for m-calpain). They are activated at pH 5.6 and inhibited by calpastatin during physiological conditions. The calpains are composed of two subunits: the catalytic portion of 80 KDa and the regulatory one of 28 KDa. The purpose of this work was to evaluate, during the first fifteen days post-mortem, the different activity of μ - and m-calpain and the expression of calpastatin in diaphragm muscle of Marchigiana cattle breed. The muscles were kept at 2°C and every two days samples were taken to perform enzymatic assays and western blot analysis. The results pointed out the presence of 80 KDa subunit of μ -calpain between 0 and 5 days post-mortem that had a reduction in the following days; 80 KDa of m-calpain showed the increased expression between 5 and 10 days post-mortem with a gradual decline in the following days. Therefore, calpastatin is present only in the first day after slaughter. The results demonstrated that the reduction of calpastatin expression is necessary to activate the calpains enzymatic system in the muscles. The μ -calpain acts on meat tenderness within the first 72 hours post-mortem, while m-calpain continues its proteolytic activity even after 6 days of slaughter. The

calpains-calpastatin system is involved in the biochemical mechanisms at the basis of meat tenderness and contributes to change muscle in meat in Marchigiana cattle breed, too.

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Effect of local by-products in the diet of heavy pig on meat quality traits

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The aim of the study was to verify the effect of two different diets on carcass and meat quality of Large Black x Nero Siciliano pigs. Conventional (COM) diet based on corn meal, barley meal and soya-bean meal and local by-products (LBP) diet (spelt meal, chestnut meal and spelt bran) were used. Diets contained 13% CP, 12 MJ/kg DE. Sixteen pigs were reared indoors in Garfagnana; the trial was performed during the finishing period and started at an average LW of 130kg. Animals were slaughtered at 190kg (T1) and 210kg (T2); DWG and FCI were calculated. At slaughtering carcass traits were determined; quality of Longissimus lumborum and samples of backfat were evaluated for chemical analysis and fatty acid profile. Data were processed by ANOVA using the GLM-SAS, significance was set at $P < 0.05$ and differences were tested by Tukey's HSD-test. Results showed higher mean value for DWG (0.48 vs 0.36) in COM; FCI was higher for LBP (8.50 vs 6.17). Carcass traits proved a higher content in backfat (15.22 vs 13.11%) and loin (10.60 vs 9.55%) in T2. Muscle showed a higher content of total lipids in COM (3.21 vs 2.23%). FA profile of the muscle showed an increase in LBP for PUFA (3.32 vs 7.43%), PUFA-n6 (8.46 vs 6.82%), PUFA-n3 (0.70 vs 0.43%), and PUFA/SFA (0.31 vs 0.25%). Backfat outer layer recorded a higher content in PUFA-n3 (1.05 vs 0.80%), HUFA (0.32 vs 0.26%) in LBP diet also inner layer produced a higher value in PUFA (13.15 vs 10.50%), PUFA-n6 (12.39 vs 9.70%), PUFA-n3 (0.98 vs 0.73%), PUFA/SFA (0.36 vs 0.28%). LBP diet presents favorable characteristics in muscle and backfat, don't show visible results in performance in the last phase of fattening; anyway the encouraging results of local by-products could be better exploited in a diet during a previous growing phase.

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Meat quality evolution of black pigs in different ages

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Due to a lack of information, studies must be done on the productive aspects of black pig from Basilicata, in relation with different factors of variability. In order to study the effect of slaughtering age on meat quality of these pigs, 12 swine were reared indoors and fed the same way. Four were slaughtered at 9 months, four at 12 and four at 15 months of age. From the carcass of every subject was taken a sample of L.D. which has been submitted to physical and chemical characterization, determined according to ASPA official methodologies (1996). The data were analysed for variance (ANOVA) by using the GLM procedure of SAS (2000). The results show that the raw meat of 15 months of age subjects, apart from having a higher pH (6.58 vs 5.14 and 5.16 respectively for 15, 12 and 9 months pigs; $P < 0.01$), are definitely ($P < 0.01$ and $P < 0.05$) redder (7.11 vs 5.35 and 3.03 respectively for 15, 12 and 9 months pigs) and softer (2.27 vs 3.30 respectively for 15 and 9 months pigs); while, the cooked meat of the subjects of 12 months of age are the softest (2.22 vs 3.38 respectively for 12 and 9; $P < 0.01$). Moreover, the raw meat of these last is less fatty (1.43% vs 1.80% and 2.92% respectively for 12, 9 and 15; $P < 0.01$). As far as concern the raw fatty acids, pigs of 9 months register the highest values of SFA (39.78% vs 36.37% respectively for 9 and 15 months pigs; $P < 0.05$), the lowest ($P < 0.05$) values of UFA (58.83% vs 61.83% respectively for 9 and 15) and MUFA (47.22% vs 50.90% respectively for 9 and 12) and consequently the highest value of the ratio $\omega 6/\omega 3$ (8.98 vs 6.77 respectively for 9 and 15; $P < 0.01$). The same trend is verified in the cooked meat, even if without statistical significance, except for the ratio $\omega 6/\omega 3$ (9.35 vs 7.22 respectively for 9 and 12; $P < 0.05$). From the extracted results, we deduced that the best productions were obtained from the 12 months of age swine.

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The influence of seaweed dietary supplementation levels on meat lamb quanti-quality characteristics

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In order to improve the quality of animal products through their enrichment in nutraceutical substances, the influence of the dietary lipid percentage replaced by seaweed on 70 day-lamb meat

production, was evaluated. Eighteen lambs "Gentile di Puglia" were weaned at 40 days and divided into three homogeneous lots: A) control; B) level 1.5%; C) level 2.5%. Performance *in vivo*, carcass quantity-quality characteristics, chemical and physical properties of meat and fatty acid profile of lipids of *Longissimus lumborum* muscle were estimated. Data were processed by GLM procedure of SAS. Results pointed out that the slaughter weight and hot yield were markedly ($P < 0.05$) higher in lambs feed with algae. The lean of control lambs thigh showed a higher statistical value: in batch B (71.0 vs 68.7%, $P < 0.05$) and especially in the lot C (67.4%, $P < 0.01$). The incidence of fat does not seem to be affected by the type of feeding. Concerning the loin, no difference in fat and bone was evidenced. Only the proportion of lean significantly varied between the two levels of integration being greater in lot B (63.2 vs 58.0, $P < 0.05$). Docosahexaenoic acid levels of B and C groups were similar but both higher than in A group (4.58-4.61% vs 0.38%; $P < 0.01$). Eicosapentanoic acid levels of B and C groups were different (1.79% vs 2.64%; $P < 0.05$) but both higher than in A group (0.46; $P < 0.01$). pH24 is greater in lot C than in the other two (5.8 vs 5.5-5.6, $P < 0.01$). As far as the colorimetric characteristics are concerned, the yellow index is higher in batch C (b^* : 11.2-11.7 vs 9.7, $P < 0.01$). Tenderness varies between 2.15 and 2.33 kg/cm² and no difference resulting from dietary treatment was found. Feeding integration with alga, changes fatty acid composition by improving the health properties of meat.

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Relationships between long chain fatty acid status of sow plasma and that of suckling piglets

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Aim of this work was to compare the blood fatty acid profile of lactating sows and their suckling piglets. Litters of three sows similar for age, parturition order and breed (Lx LW) and inseminated with the same Landrace boar semen were used. During lactation dams were housed in individual boxes and fed a concentrate for lactating sows. The trials started seven days after parturition and lasted four weeks. Each week one piglet per litter was stunned, exanguinated and blood samples were collected. Sow blood samples were collected weekly during the experiment. Fatty acid analyses on blood and feed samples were performed by gas-chromatograph. Data were analyzed by ANOVA, to detect differences in FA composition between progeny and dam blood during the weeks of the suckling time. Sow diet contained about 3% of lipids, the linoleic acid represented more than 50% and C18:3 only about 4% of total FA. The FA profile differed markedly between piglets and sows. The SFA was significantly higher in piglets, due to the higher proportion of C16:0 and C18:0. PUFA-n3 were found only in traces in sow blood, whereas their concentration was more than 1% in piglet

blood. PUFA-n3 in piglet was represented by alfa-linolenic acid and their long chain FA derivatives such as DPA and DHA. The proportion of PUFA-n6 was significantly higher in sows due to the higher values of linoleic acid. The content of the essential FA (alfa-linolenic and linoleic acids) in the sow blood is explained by FA composition of the diet. The presence of very long chain fatty acids (> C20) only in piglets blood suggests an active desaturase and elongase enzyme ability in their tissues. The FA composition in blood of sows and piglets did not vary significantly between weeks. This could be explained by the strong relationship between FA profile of the diet and that of the most body fat reserves mobilized by lactating sows.

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Effect of cooking on the fatty acid composition and lipid oxidation of Sarda suckling lamb from different rearing system

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The aim of this work was to study the effect of cooking on the fatty acid composition and lipid oxidation of meat from Sarda suckling lamb. Forty-eight suckling lambs (24 males and 24 females) were divided into 2 groups subjected to different feeding system: 24 raised indoor (Group IN) and 24 followed their mother outdoor on pasture (Group OUT). Lambs were slaughtered at 28 days of age. After 24 h of refrigeration at 4°C, the tight muscles were dissected from each right half-carcass and split into pieces; a 50 g portion of each sample was cooked in a microwave oven at 650 W for 35 s to a final internal temperature of 75°C. Chemical analysis, fatty acid composition and TBARS were measured in raw e cooked samples. Data were analyzed by one-way ANOVA with rearing system as fixed factor. One-way ANOVA was used to compare raw versus cooked samples. Cooking did not produce changes in total lipid and ash content but induced significant changes ($P<0.01$) in the values of moisture and protein content in meat for both rearing groups. The feeding system of lambs did not modify almost all FA analyzed. The cooking modify several of FA considered. The cooked meat has a significant higher level of PUFA n-3 (1.80 fold), a better value n6/n3 and lower levels of SFA compared to the raw meat of both feeding system. Lipid oxidation, expressed as mg MDA/kg muscle, was not influenced by rearing system. The MDA was increased significantly by cooking on both experimental groups. In cooked samples, the MDA was higher in Group OUT compared to Group IN ($P<0.01$), probably due to the higher proportion of polyunsaturated fatty acids. The rearing system of lambs did not cause substantial changes in FA composition and lipid oxidation stability. The cooking cause marked changes in FA profile and lipid oxidation even if the values of MDA detected in cooked

samples of both experimental groups are very low and within the limits of rancidity indicated by literature in 1-2 mg MDA/100 g of product.

Acknowledgement

The research was funded by University of Sassari (F.A.R. grant)

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Effect of diets with different nutritional value on growth performances in Nero Siciliano pigs

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This study was undertaken to provide information of the influence of feeding level during the period prior to fattening on growth, carcass traits and fatty acids profile of subcutaneous backfat of Nero Siciliano pigs finished under free-range conditions with acorns and grass. During the period prior to fattening, a group of 40 pigs balanced for sex (barrow and gilt), live weight (29.6 ± 0.14 kg) and age (6 month old) were equally distributed in two dietary groups of 20 pigs each: high feeding level group (H) was fed with 90 g feed kg^{-1} LW 0.75 (live weight) and moderate low feeding level group (ML) was fed with 70 g feed kg^{-1} LW 0.75. The baseline weight of each pig was recorded and the weight was measured every 15 days to determine the average daily gain (ADG); ultrasonic measurements of backfat was taken every month for all pigs at the level of the last rib; backfat biopsy samples were taken at the beginning of the fattening periods (188 days after the trial start) and the acidic composition determined; each fatty acid was carried out by gas chromatography analysis GC-FID. Data were subjected to ANOVA. The pigs ADG prior to fattening were: 392.8 and 328.4 ($P<0.0001$) g day^{-1} and the weights were: 103.1 and 91.9 ($P=0.0002$) kg in H and ML pigs respectively. Feeding level during the period prior to the fattening had significant effect on inner backfat layer thickness ($P=0.047$). The subcutaneous backfat from H pigs had a significantly higher proportion of C18:1 n-9 ($P<0.005$) and \sum MUFA (monounsaturated) ($P=0.025$) acids than those from ML pigs while the ML pigs showed a higher proportion of C18:2 n-6 ($P=0.0004$), C18:3 n-3 ($P=0.016$) and \sum PUFA (polyunsaturated) acids than H group. In conclusion, a marked effect of feeding system was observed on pig productivity and fatty acid composition.

Acknowledgement

Research supported by MIUR, PRIN-2008 Prof. V. Chiofalo

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Chemical, instrumental, sensory and microbiology characteristics of goat meat sausages and ham

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In order for the meat goat producer to survive, new avenues for marketing goats must be created. Quality of food products is determined by their sensory attributes, chemical composition, physical properties, microbiological characteristics, shelf life, packaging and labeling. Cooked ham is one of the most popular processed meat products with value-added. Its quality is influenced by many factors (meat cut, brine, the rate and extent of tumbling or massaging, and the cooking time and temperature) and it is judged by various sensory characteristics. Chemical composition, texture profile analysis and *E. coli*, *Salmonella sp.*, *S. aureus*, fungi and yeasts in goat meat sausages and ham were evaluated. Commercial pork sausages and ham were used as control. Sausages and ham cylinder samples (2 cm high, 2 cm diameter) were compressed twice to 50% of their original height (compression platen of 50 mm in diameter). Force time curves were recorded at a crosshead speed of 50 mm/min. Hardness, cohesiveness, elasticity, gumminess and chewiness were evaluated. Sixty-eight untrained panelists performed sensory evaluation, through a level of acceptance testing, using a 5-point hedonic scale. Protein and fat in goat meat sausages and ham were 19.0:8.9% and 25.1:2.1% respectively; in relation to sausages and ham control the values were 19.0:14.4% and 22.9:3.7% respectively. Cholesterol in goat sausages and ham was 54.2 and 35.7 mg/100g respectively; in commercial pork products these values were 42.6 and 63.5 mg/100g respectively. Saturated (SFA) and polyunsaturated fatty acid (PUFA) in goat sausages and ham showed 28:38% and 45:27% values respectively. In pork products these values were 33:23% and 36:22% respectively. Goat ham PUFA's showed a higher value ($P<0.05$) to relation pork ham PUFA's. Goat sausages showed a higher value ($P<0.05$) of hardness, gumminess and chewiness than commercial pork sausages. Ham goat registered a higher value ($P<0.05$) of elasticity and chewiness than control ham. Panelists detected no differences ($P>0.05$) in color, flavor, odour, and texture between goat and pork hams. Color, odour and texture in sausages the consumer panelists detected no differences ($P>0.05$). However, the flavor in sausages was similar ($P<0.05$) to sausages control. *E. coli*, *Salmonella sp.*, *S. aureus*, fungi and yeasts were not detected in sausages and ham. The goat meat products were rich in protein, with a good lipid profile. However, texture characteristics and level of satisfaction, were not as good as commercial products.

149F

High density canine 170,000 SNPs array for recessive trait mapping in a canine pure breed

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The present study aims to evaluate the basis of a motor-sensory neuropathy, recently reported in Rhodesian Ridgeback (RR) dogs and clinically similar to a group of human diseases known as Charcot-Marie-Tooth (CMT). In literature about 40 clinical subtypes are reported, all of them are inherited with variable transmission. In RR hereditary defects of development of Peripheral Nervous System were reported making this breed a possible animal model for comparative study of CMT. A family group of RR was reported to show tremors and fasciculations, limb muscle degeneration, atrophy, demyelination and myelin outfolding. The pathological cases showed clinically and histological characters very similar to three subtypes of human CMT: *CMT4B-1*, *CMT4B-2* and *CMT4H* (autosomal recessive inheritance). Causative genes for these forms of the disease are identified as *MTMR2*, *MTMR13* and *FGD4* respectively. Biological samples of three healthy dogs and three affected ones, all belonging to the same family group, were collected. Genomic DNA was extracted and parentage was verified comparing the genetic profiles using a panel of STRs internationally recognized. The human genes *MTMR2*, *MTMR13* and *FGD4* on dog genome nucleotide sequence assembly were identified and the homology and conservation were evaluated. Genome-wide Association (GWA) approach by 170,000 high-density SNPs DNA microarray (Illumina) to map potential candidate genes for CMT disease were used. Seventeen candidate regions were found using Homozygosity Mapping. The described method has been useful to identify the common regions in affected RR, anyway the results could be further on improved increasing sample size with subjects new blood lines.

150F

Prevalence of MYBPC3 gene mutations and association with hypertrophic cardiomyopathy in several cat breeds

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Pure breed cats are a useful model in inherited disease study due to the specific knowledge of population genetic and accurate genealogical data often tracing back to the foundation stock of the breed. Furthermore high conservation rates with human and domestic animals genomes are well known. The aim of this work was to examine the Maine Coon (MC) and Ragdoll (RD) HCM-associated *MYBC3* known mutations in a large sample of cat breeds, and to investigate the genotype-phenotype association. An *in silico* evaluation of the impact of the amino-acid (aa) substitutions was carried out. 762 cats (MC, RD and other 9 breeds) were genotyped at loci *A31P* and *R820W*, cats were examined with echocardiography (Osservatorio Italiano HCM Felina). Distribution of the clinical form in all breeds and disease Odds Ratio in MC were determined. The potential impact of the aa substitutions was simulated using Modeller 9v8 and the human *MYBPC3* as template. Data strongly confirm *A31P* (all. freq. 0.23) and *R820W* (all. freq. 0.17) as private mutations of MC and RD respectively. In MC significant differences ($P < 0.001$) were recorded in the genotypic frequency of homozygous *A31P* mutated between healthy and affected (OR 9.5, CI 4.9-65.7). Functional modeling predicted the potential ability of *A31P* to perturb the overall fold and stability of the protein. However the *A31P* heterozygous status is not fully associated with HCM, suggesting a not complete dominance and other mechanisms involved in HCM development. These data will help the cat breeders to understand HCM genetic test results and to drive their selective choices.

151F

Genomic analysis for the valorization of an Italian local swine breed

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Recent challenges to the EU agricultural sector call for new management approaches that reconcile animal food production with the conservation and sustainable use of biodiversity, promotes the delivery of ecosystem services and benefits the agricultural sector and society as a whole. Despite the admirable efforts made in the last years, one of the main obstacles in recovering pig biodiversity lies in the limited number of Italian local breeds able to withstand the commercial competition with cosmopolitan breeds (i.e. the Large White, Landrace and Duroc) and the commercial crosses. To date in the national Herd Book only six local breeds are recorded: Cinta Senese, Mora Romagnola, Nero Siciliano, Casertana, Apulo-

Calabrese and Sarda. In recent years, an attempt has been done to reconstruct an ancient genetic type barely still existing, the “Nero di Garlasco”, characterized by a black coat colour and growth rates similar to the ones of commercial breeds, in the province of Pavia (Lomellina). To achieve this goal, a genetic characterization of the residual population should be conducted. The tight bottleneck this population passed, allowed the fixation of genetic markers enabling a precise traceability of its products, ideal requisite for the valorisation of the production system. Thanks to innovative technologies, a population of 96 animals, belonging to the Nero di Garlasco genetic type, was analyzed using the PorcineSNP60 BeadChip, in order to screen about 60,000 SNPs. The obtained data allowed a first description of the genetic structure of this population, but further studies are required to characterize this swine genetic type.

152F

Association analysis of candidate SNP with milk traits in Italian Holstein cows

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Thanks to the availability of high-throughput genotyping technologies, SNP became the genetic markers of choice for high resolution genetics and genome-wide association studies. The estimate of the effects of SNP on production traits has to be verified in any new population in which this information is planned to be used in marker assisted selection. In the first step of a project supported by “Accordo Quadro CNR- Regione Lombardia” a 124 SNP panel was selected to genotype loci involved in milk protein and fat biosynthesis and responsible for genetic disease susceptibility in Italian Holstein breed. Also 13 SNP useful for animal identification and paternity analysis were included in the panel. About 500 cows were collected included both unrelated animals and family trios in order to confirm the described association between SNP and milk traits (milk yield, protein and fat yield and percentage), to check its functionality in paternity analysis, and to find new associations with other phenotypic traits, such as milk fatty acids (FA) composition. DNA was extracted both from blood and milk and genotyped with the Illumina GoldenGate Assay. Of the 124 SNP submitted for scoring by the Illumina assay design tool, 96 (84 for milk protein and fat biosynthesis and 12 for paternity analysis) had a SNP score guaranteeing a successful assay. Cervus software was used for parentage analysis. The SAS GLM procedure was used for association analysis with milk quality traits. Significant association

were found with some SNP in the casein genes and genes involved in fat metabolism. Their use in marker assisted selection programs could be useful to modify nutritional qualities of milk through genetic selection.

153F

Investigation of the 5' non coding region of the ACACA gene in sheep

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Transcription of the *ACACA* gene, which encodes the flux-determining enzyme in the regulation of fatty acid synthesis in animal tissues, is initiated from three promoters: PI transcripts are abundant in adipose tissues and mammary gland; PII have ubiquitous tissue distribution; PIII play an important role in lipogenesis during lactation. The objective of the present work was to investigate the variability of the three *loci* in sheep. The DNA of 65 Altamura, 63 Gentile and 48 Sarda sheep was amplified at the following loci: from 4263 to 4651 bp and from 9469 to 9919 bp of the *Ovis aries ACACA* gene (accession AJ292285); from 2811 to 3200 bp of the *Bos taurus ACACA* gene (accession AJ312201). Amplicons were directly sequenced and were blasted in the GenBank database against the whole genome shotgun sequence of *Bos taurus* chromosome 19, accession NC_007317, to assess their distance from the starting codon of the *ACACA* gene, which resulted by over 25,000 bp for PI and PII, while PIII fell within the long intron 1, about 17,000 bp after the start of exon 1 and 5000 bp before the start of exon 2. In each of the three amplicons, three point mutations were detected; the *in silico* analysis performed using MatInspector software (<http://www.genomatix.de/>) showed that only two of them fell within putative binding sites for transcription factors: the first, located in PII, is a signal transducer and activator of transcription; the second, located in PIII, is a B-cell-specific activator protein, a member of the paired box domain gene family that encodes nuclear transcription factors important in development and cell proliferation. Allele frequencies of the mutations in the Gentile (for PII locus) and the Altamura (for PIII locus) highly differ ($P < 0.0001$) from the frequencies of the other two breeds; the observed evidence of genetic heterogeneity, at two loci of the same gene, allows the hypothesis that the two loci encode sequences related to peculiar traits of the local breed, and deserves further investigation.

154F

The sequencing of Water Buffalo genome

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The sequencing of the buffalo genome is the primary purpose of a project that was started by an Italian consortium composed of the following research institutions: Parco Tecnologico Padano, IBBA-CNR, ISPAAM-CNR, CRA-PCM, CASPUR, IZSM, ConsDABI, University of Naples, University of Piacenza, University of Viterbo, University of Molise, University of Sassari. The buffalo genome project has three components: 1) *de novo* sequencing, assembly, annotation, and transcriptome analysis, 2) variation analysis and the creation of a SNP panel, and 3) comparative sequencing and analysis of wild relatives. Two different platforms were used for sequence production by USDA, to avoid bias and to obtain the very long insert libraries, necessary for the *de novo* assembly. With Illumina Genome Analyzer II sequencing: 3 paired end short insert (350-500bp) libraries and 6 mate-pair libraries (~5kb inserts) were created. With Roche 454 sequencing: two whole genome shotgun libraries (WGS) and 5 long paired end (LPE) libraries with 20kb inserts were constructed. The total sequence yield was 137Gbp with all the data combined: raw sequence coverage from Roche 454 reads was about 1.6X and from Illumina 45.6X. After data processing for quality, which is necessary for proper genome assembly, we have more than 22X depth in sequence coverage and about 79X in clone coverage (distance coverage by unique long insert ends) which is a high coverage considering that the coverage for Bovine genome was 7.1X (www.ensembl.org). The University of Maryland are leading the sequence assembly using Celera software in collaboration with CASPUR. Annotation is being led by EBI using the Ensembl pipelines in collaboration with Italian groups. An expression atlas of the buffalo was also created, data from which provide information to aid annotation. The variation detection and the setup of a SNP panel is underway using DNA samples from river and swamp buffaloes. Completion of the genome sequence will help to apply innovative technologies for breeders and farmers, i.e. high throughput SNP detection, to improve reproductive performance and production potential in water buffalo.

155F

Effect of polymorphisms in the goat *DGAT1* gene on milk fat traits

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Acyl-CoA:diacylglycerol acyltransferase 1 (*DGATI*) plays a key role in lipid metabolism, catalyzing the final stage of triacylglycerol synthesis. In cattle, a non-synonymous substitution in *DGATI* influences enzyme activity with a major effect on milk composition. This aspect makes *DGATI* an interesting candidate gene to explain the phenotypic variation of milk related traits in other species such as goats. Aim of this work was to identify polymorphisms in the goat *DGATI* gene and to study their association with milk fat and other milk related traits. The goat *DGATI* gene was screened for polymorphism in 12 animals of 6 breeds. Sequence analysis of the gene from exon 3 to 17 revealed the presence of 4 single nucleotide polymorphisms (SNPs) in introns 7, 12, 14 and 16. No SNP was identified in the coding regions of our sequences, confirming a low level of polymorphism of this gene in goat. The SNPs genotyping, of 109 Alpine and 84 Saanen bucks, revealed high frequencies of the major alleles (from 0.82 to 0.99). To evaluate the effect of single SNPs an association analysis on about 28.000 records of milk (kg/d), fat (kg/d and %) and protein (kg/d and %) of 1900 daughters was performed using a mixed linear model, with bucks genotype (from 59 Alpine and 55 Saanen bucks) as fixed effect among others, followed by permutations. Results showed significant associations of the SNP in intron 12 with fat % in the Alpine breeds, and of the SNP in intron 14 with fat kg/d and % in the Saanen breed.

Acknowledgment

This research was supported by Mipaaf (project SelMol)

156F

Accounting for additive genetic relationship in genetic evaluation of Burlina breed

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The aim of this study was to develop a conservation index for the Burlina cattle breed, combining the average coefficient of kinship (k-coefficient) of each individual, with the rest of the population, and the estimated breeding value (EBV) for production. This would allow the identification of animals that can ensure an improvement of production in the following generation without compromising the genetic variability of the population. Burlina is a dairy cattle breed reared in the North-East part of Italy, particularly in Treviso province. The number of reared animals decreased during the years, and programs to safeguard Burlina were set up since 1980s and currently about 300 cows are involved in the milk recording scheme. Monthly test-day records and pedigree information were provided by the Breeders Association of Treviso

Province. Data to calculate f-coefficient included 2374 animals, and 14,862 milk yield records collected on 704 cows in 10 herds from 1999 to 2010 were available for the prediction of EBV. The k-coefficients were calculated using the INBREED procedure of SAS (2006), and EBVs for milk yield were obtained using a single-trait animal model and REML procedure. The model accounted for herd-test date, days in milk, age at calving within parity, and month of calving as fixed effects, and animal, permanent environmental and residual as random effects. The k-coefficient and EBV were weighted in a conservation index using three pairs of values, namely 20:80, 50:50 and 80:20, where the first given more weight to k-coefficient and the last to EBV. Comparing results will be described. This conservation index can be used as a tool for planning breeding schemes controlling the future rate of additive genetic relationship without penalize the genetic merit of individuals.

157F

Analysis of milk coagulation properties and non-coagulation probability in test day records

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Aims of this study were to investigate the effects on milk coagulation properties (MCP) traits and probability of non-coagulated samples (NC). The MCP is measured as a combination of milk coagulation time (RCT, min) and curd firmness (a_{30}) while NC occur when the sample doesn't coagulate in 30 min. From May to December 2010, individual milk samples from 2223 Holstein Friesian dairy cows were collected during test day recording. The animals were daughters of 93 bulls and reared in 192 herds in Veneto region. Samples were preserved with the addition of bronopol and storage until the analysis. The samples were analyzed in Veneto Regional Breeders Association laboratory from 0 to 6 days after collection for milk contents and MCP traits. The MCP analysis was performed with lattodinamografo (Foss-Italia, Padova, Italy) using Hansen standard rennet with a rennet activity of 0.051 IMCU/mL of milk. All collected data were then statistically processed for NC probability through Logistic procedure and for RCT and a_{30} of coagulated samples with GLM procedure in SAS. All models included the effect of: milk yield; content of fat, protein and somatic cell score (SCS); class of age of sample, class of month of test day, sampling in morning or evening milking and sire. Thirty-one percent of samples were NC. In all models fat content, SCS and sire were significant ($P < 0.001$). The NC probability was affected also from class of sample age and class of month of test day, while a_{30} from protein content and month of test day.

158F

Analysis of amylase gene in wild and domestic species

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Starch is one of the most important glycemic carbohydrate components in foods and its nutritional property is related to its rate and extent of digestion and absorption in the small intestine. Dietary readily digestible hydrolyzable carbohydrates (hCHO) are broken down in the small intestine of non-ruminant species by pancreatic α -amylase. In particular in horse, experimental and epidemiological evidence suggests that high consumption of starch is an important risk factor for colic, the major cause of equine mortality. The digestive physiology of the horse and its ability to adapt to carbohydrate load are therefore of vital consequence. Moreover in piglets starch digestibility is highly variable, and it has a crucial role in the post-weaning growth-check commonly observed, resulting from a period of underfeeding along with a number of environmental and psychological stresses, which can greatly compromise the overall growing and finishing performance of the animals. Aim of this work has been the analysis of the amylase genes in these two domestic species to identify interesting polymorphisms useful to evaluate genetic variability of this genes, making a comparison of the animals analyzed between different breeds and in pig also with wild board. The amylase genes in mammals is expressed mainly in pancreas and in salivary glands, thus we have to distinguish at a genetic level these two types of amylase genes. In molecular databases we found the sequences of amylase genes for horse, but little information about this genes were found in pig. RNA has been extracted from pancreas and salivary glands tissues from both species. After a retrotranscription to obtain the cDNAs, we have been able to confirm the distinction between the two amylases in horse and to found out that in pig. The results of SNP discovery on genomic DNA are presented.

159F

Genetic diversity of Akhal-Teke horse breed in Italy assessed by molecular markers

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The Akhal-Teke is a horse breed of Turkmen origins, from the southern region of the Turkmenistan modern country. These horses are well known for their speed and endurance on long marches.

These "golden-horses" are adapted to severe climatic conditions and are thought to be one of the most ancient surviving horse breeds. Since 1998 the Laboratory of the Department of Animal Science of the University of Milan tested this rare breed in Italy for parentage control using both classical markers (blood groups and protein polymorphisms) and microsatellites. During this period blood samples from 102 horses were collected. In the present study, the mitochondrial D-loop DNA region (mtDNA) genetic variability and haplotypes relationship of Akhal-Teke horses reared in Italy were investigated. Twenty-two unrelated horses belonging to different maternal lineage from our database were chosen. The mtDNA was extracted using standard procedures. A 379 bp fragment of horse mtDNA was amplified and sequenced. The obtained sequences were aligned among themselves and with the complete horse mtDNA sequence (GenBank# X79547), using the CLUSTALX software. Diversity parameters, haplotype diversity (Hd), nucleotide diversity (π) and average number of nucleotide differences (k) were calculated using DnaSP 5.1 software. Horse sequences from GenBank database were used for relationship investigations. Median-joining network and mismatch analysis were calculated using NETWORK 4.5.0.1. Twelve haplotypes were found in the Akhal-Teke horses reared in Italy. Hd, π and k values were 0.938, 0.021 and 6,448 respectively, indicating a good variability. The comparison with database sequences showed the presence of seven haplogroups.

160F

Differences in *DGAT1* gene expression associated with gene polymorphisms in Gentile di Puglia, Altamura and Sarda sheep

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AcylCoA:diacylglycerol acyltransferase (*DGAT1*) plays a key role in triacylglycerol synthesis. In cattle, the *DGAT1* gene contains Lys232Ala that explains 50% of the genetic variation in milk fat percentage. Moreover, Scatà *et al.* (Animal Genetics, 2009) found SNPs in *DGAT1* ovine gene and demonstrated that a novel detected SNP in the 5'-UTR gene explains a variation of milk fat content in the Sarda sheep. In order to determine the mRNA level differences associated with polymorphism in the gene, gene expression in different sheep breeds, with different *DGAT1* alleles was investigated. Starting from the results presented in Scatà *et al.*, milk samples from an experimental population genotyped for the *DGAT1* gene, namely 9 Altamura, 4 Gentile di Puglia and 5 Sarda sheep, were collected. Total RNA was isolated from milk somatic cells and Real-Time PCR was carried out by amplifying *DGAT1* and the ref-

erences kappa casein (*CSN3*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Using both reference genes to calculate *DGAT1* gene expression level, similar results were obtained ($r = 0.8$). Since one of the two reference genes used was specific for mammary gland cells (*CSN3*), from the high correlation found it can be said that the cell types present in the milk samples used in this work resulted to be mainly mammary gland cells. No direct relation between gene polymorphisms and mRNA level has been found. The comparison of the results obtained with *CSN3* and *GAPDH* resulted a suitable method to ascertain the nature of the cell recovered from milk. In the further investigation it will be used in expression vector in order to check the influence of A allele in the promoter functionality.

161F

Preliminary results of the myostatin promoter analyses on young Marchigiana sires

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The Associazione Nazionale Allevatori Bovini Italiani da Carne (ANABIC) periodically carries out performance tests on Marchigiana young bulls to assess their breeding and morphological values, and therefore reject or approve them for natural or artificial insemination. In addition to the classical morphological and genetic parameters, ANABIC tests all the young Marchigiana sires also for the presence or absence of the SNP causing the “double muscling” trait. It has been a year since the Applied Biology Department lab (University of Perugia) started genetic tests in order to reveal two additional SNPs (T/A at -371 and G/C at -805) within the promoter region of the myostatin gene. The SNPs are revealed by a PCR-RFLP-based technique, since such mutations introduce a further restriction site in addition to those already present within the promoter. A total of 62 young Marchigiana sires have been tested so far. Regarding the SNP T/A a 56,4% animals were homozygous T/T, a 43,6% animals were heterozygous T/A whereas no homozygous A/A animals were found. With regard to the SNP G/C an impressive 96,8% animals were homozygous G/G, only a 3,2% animals were heterozygous G/C while no homozygous C/C animals were found. The results of a previous funded project (SELMOL) hypothesised that these SNPs could be linked to the modulation of the muscular traits expression. It will be thus mandatory to carry out some correlation studies on these young bulls to confirm this important link between SNPs and productive traits.

162F

The first chicken non-official comparison test

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The first meeting of a non official (not financially supported by ISAG) working group for chicken microsatellite genotyping comparison test was held during the 32nd ISAG Conference. The aim is to establish a network of labs working with STR for chicken genetic studies. Secondary goal was to supply reference DNA samples to labs interested in participation, support for biodiversity studies of autochthonous breeds using the same techniques, and production of a related database. The trial was run by 7 labs on 29 shared DNA samples of different chicken breeds using a various number of microsatellites (from 5 to 28). The results from an initial comparison of genotype data were encouraging, with a high percentage of correct allele scoring among labs (>90%). The resulting discussion suggested formation of a group composed by all the labs working on chicken biodiversity in order to share DNA samples and all the information about analysis of genetic diversity. Concluding, the group suggested listing all the studied breeds, all employed markers, and sharing information on the biological samples available from different labs. As SNPs become more and more popular in genetic studies of farm animals (effective cost and processing ease), the group would also consider them as an option for future biodiversity studies in chickens.

163F

Mitochondrial DNA variation in the Maremmano horse breed: new genetic insights of an ancient Etruscan connection

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The Maremmano is an Italian warmblood horse mostly bred in the provinces of Grosseto and Viterbo (Central Italy). Today's records in the Maremmano's studbook could be traced back to 4 stallions and 440 mares. It is believed that the origin of this breed goes back to Etruscan horse populations which were crossbred with modern stocks in the last centuries. We evaluated the nature and extent of variation of the mitochondrial gene pool in Maremmano horse, following the approach recently used to analyze humans (modern Tuscans) and bovines (Maremmata and Chianina breeds). After an accurate genealogical analysis of the maternal lines, DNA samples were collected from 73 unrelated animals. All mtDNAs were sequenced from np 15,491 to np 16,100, thus including more than half of the control region (nps 15,469-16,660). We used an adapted version of mtPhyl (<http://eltsov.org/mtphyl.aspx>), which was initially designed to analyze human mtDNA sequences, to classify the detected 44 unique haplotypes into 12 different haplogroups. The most frequent haplogroup is represented by the ancient and widespread equine lineage A1, but of perhaps greater interest, we also found a high incidence (>10%) of haplogroup C1, which is characteristic of Middle Eastern breeds and barely found among European horses. The peculiar presence of this eastern lineage would generally describe a parallel genetic history connecting the origin of the Maremmano breed to the ancient Etruscan population. Obviously, this fascinating hypothesis could only be confirmed after a further detailed phylogeographic analysis of such lineages, performed at the highest level of molecular resolution – that of complete mtDNA sequencing – as previously performed for human and bovine lineages.

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Survey on the distribution of fat globule size of Friesian and Jersey cows in Lombard dairy farms

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Globules size influences the visco-elasticity of the clot and the texture of the cheese. From a nutritional point of view, the mean diameter of globule is negatively correlated with the content of phospholipids. Moreover, the globules size and the membrane/volume ratio influence the lipolysis process in cheese, with implications on the final product aroma. The aim of this work was the study of fat globules size and distribution in Lombardy cows. Eight lombard dairy farms were monitored: individual milk samples were collected monthly during one year period. Milk of 59 Friesian cows from all the farms and 7 Jersey cows from a single farm were sampled. All the cows were half sisters with 6 common fathers for Friesian and 3 for Jersey, chosen among the best breeding bulls selected in Italy. The particle size analyses of fat globules were performed using a granulometer Mastersizer 2000 (Malvern instruments Ltd., UK) with a laser source at 633 nm. The study showed a variation of the Sauter Mean Diameter from 2.1 μm to 4.4 μm for Friesian cows and from 3.5 μm to 4.8 μm for Jersey. The analysis of variance showed significant differences depending both on farms and on fathers. For the Friesian, the *post hoc* analysis showed at least 3 significantly different groups in farms and at least 2 significantly different groups between half sisters. For the Jersey, 2 significantly different groups among half sisters were found. The differences among the farms could be used in planning the collection of milk in function of the technological destination. The differences among the breeding bulls could be useful for a targeted selection of animals according to globules size. Further studies will be directed to the technological characterization of milk with different fat globule dimensions.

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Effects of phytase on growth performance, nitrogen and phosphorus balance in the heavy pigLuca Malagutti, G. Matteo Crovetto, Mario Pirondini,
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The effects of a commercial phytase (Ronozyme® NP) were studied in the fattening pig. In comparison with a control diet (C) which included 0.5% CaHPO₄, two diets with no CaHPO₄ were tested: F1 and F2, with 0.1 and 0.2% Ronozyme® NP, respectively. Sixty Landrace x Large White castrated male pigs of about 47 kg BW were randomly divided into 3 groups of 20 animals; the pigs of each group were splitted into 5 pens of 4 animals each. Pigs were individually weighed to calculate the average daily gain (ADG) and feed intake was registered per each pen, to compute the feed conversion ratio (FCR, kg DM intake/kg weight gain). The trial was

divided into 2 phases: 47-99 and 100-140 kg BW. At about 120 kg BW, 6 pigs per diet were housed in individual metabolic cages for the balance trial. All data were analysed by the Mixed procedure of SAS package. Phytase in the feed resulted in higher ADG (723, 783 and 772 g/d for C, F1 and F2, respectively; $P=0.048$) and improved FCR (2.84, 2.64 and 2.65; $P=0.030$) in the first phase. No difference was registered in the second phase, whereas the total trial shown a better FCR (3.05, 2.94 and 2.93 for C, F1 and F2, respectively; $P=0.050$), and a trend for a higher ADG (774, 806 and 805 g/d; $P=0.058$). Digestibility (%) was similar for all diets (among others, on average: DM=88.5, En=88.9, CP=87.4). Dressing proportion at slaughter was similar for the treatments (0.828 on average) as well as the other slaughter parameters. Total N excretion was not different between the dietary treatments: on average 52% of the intake N. P excreted for C, F1 and F2 was 4.7, 4.3 and 4.3 g/d, respectively ($P=0.477$); expressed as % of the intake P, it was 47.1, 53.3 and 52.5, respectively ($P=0.356$). In conclusion, phytase was effective in improving growth performance until 100 kg BW at both inclusion levels in the feed. For heavier animals it did not improve digestibility nor N and P balance.

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The Scientific and the Organizing Committees of the 19th Congress of Animal Science and Production Association gratefully acknowledge the following individuals who have served as reviewers.

Abeni Fabio
Ajmone Marsan Paolo
Bernabucci Umberto
Biagini Davide
Biondi Luisa
Bittante Giovanni
Canavesi Fabiola
Capelletti Maurizio
Cassandro Martino
Catillo Gennaro
Contarini Giovanna
Dall'Olio Stefania
Della Casa Giacinto
Di Francia Antonio
Federici Claudia
Finocchiaro Raffaella
Fronte Baldassare

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Pais Antonio
Panella Francesco
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Piasentier Edi
Pilla Fabio

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Polidori Paolo
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Speroni Marisanna
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