Fatty acid composition of the intramuscular fat in the longissimus thoracis muscle of Apulo-Calabrese and crossbreed pigs

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Highlights

- The aim was to compare muscle fatty acid composition between two pig genetic types
- Apulo-Calabrese pigs showed slightly higher contents of monounsaturated fats.
- Crossbreed pigs had slightly higher amounts of polyunsaturated fatty acids.
- The two genetic types show similar loin fatty acid profiles when reared indoor.

Fatty acid composition of the intramuscular fat in the *longissimus thoracis* muscle of Apulo-Calabrese and crossbreed pigs

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Abstract

The aim of this study was to investigate the fatty acid profile of the *longissimus thoracis* muscle in two genetic types of pigs. Fifty one pigs of the Italian local breed Apulo-Calabrese and 52 crossbreed [Duroc x (Landrace x Large White)] were fed the same commercial diet and reared in the same indoor system. Fatty acid composition was assessed through Folch method and the obtained data were analysed with a mixed model to identify possible differences between the two genetic types. The Apulo-Calabrese pigs showed significantly higher contents of heptadecenoic acid ($P< 0.0001$), myristic ($P= 0.03$), arachidic ($P= 0.04$), myristoleic ($P= 0.004$), palmitoleic ($P= 0.01$) and gondoic ($P= 0.01$) acids. On the other hand, crossbreed samples presented higher contents of docosahexaenoic ($P= 0.01$) and arachidonic acid ($P= 0.01$). Except for heptadecenoic acid, there were no great differences in *longissimus thoracis* muscle fatty acid profile between the two genetic types, suggesting that when Apulo-Calabrese pigs are managed in the same rearing conditions as crossbreeds their *longissimus thoracis* muscle fatty acid composition is similar.

Keywords: pig, breed, Apulo-Calabrese, intramusculat fat, fatty acid, crossbreed pig

1. Introduction

Fatty acid (FA) composition has profound effects on the organoleptic properties and nutritional value of meat with regards to human health. However, technological requirement by the food industry and dietary demands by consumers do not completely match. In particular, pork processing industry requires meat with a limited amount of polyunsaturated FA (PUFA) as they are more likely to incur in lipolytic and oxidative processes, causing rancidity, abnormal flavours, fat softness and altered organoleptic properties (Wood et al., 2008). On the other hand, high proportions of monounsaturated FA (MUFA) and omega 3 (*n*-3) PUFA have been reported to have beneficial effects on consumers' health (Briggs et al., 2017). Recently, there has been an increasing consumer interest in niche products derived from local pig breeds (Pugliese and Sirtori, 2012) due to their high added value and eating quality. Among the Italian local pig breeds listed in the national herd book is Apulo-Calabrese, from the Calabria region. This breed is characterized by reduced growth and carcass performance, but its meat has quality features suitable for the production of Protected Designation of Origin (PDO) salami (Micari et al., 2009) which is well appreciated not only in Italy but also abroad. This breed is well adaptable to different production systems (Micari et al., 2009; Pugliese and Sirtori, 2012) but is often reared outdoor, which has a negative impact on the environment (Acciaioli et al., 2012). FA composition of pork is affected by the animal genetics (Wood et al., 2008) and local breeds are generally reported to have a higher propensity for fat deposition. However, most literature in the scientific domain compares the fatty acid profiles of pigs belonging to local breeds reared outdoors, with highly selected pigs reared under the intensive system. This environmental variability makes it difficult to verify the real difference between the two genetic types.

The aim of this study was to compare the FA profile of Apulo-Calabrese and crossbreed pigs reared indoors and fed the same commercial diet, in order to identify the effects of the genetic type over muscle FA synthesis and storage.

2. Material and Methods

All procedures performed in this study were in line with the Italian and European legislation concerning the protection of animals kept for farming purposes, their transport and their slaughter procedures, and therefore did not require further specific authorization. Slaughter was performed under the control of the Veterinary Service from the Italian Ministry of Health.

2.1 Animals

For this study, 51 Apulo-Calabrese pigs (45 gilts and 5 barrows) registered in the herd book of National Pig Breeder Association (ANAS) and 52 [Duroc x (Landrace x Large White)] crossbreed pigs (24 gilts and 26 barrows) were used. All the animals were free from the deleterious alleles of the *Protein Kinase AMP-Activated Non-Catalytic Subunit Gamma 3* (*PRKAG3*) and the *Ryanodine Receptor 1* (*RYR1*) genes (Aboagye et al., 2018). The pigs were reared in the indoor system on the same farm and were fed the same commercial diet (Supplementary Table 1) in a liquid feeding system with dry feed and water mixed in a 1:4 ratio. At the end of the trial, the pigs were transported to a local processing plant and slaughtered after being electrically stunned by tongs (head only; 220 V, 1.3 A). Apulo-Calabrese pigs were slaughtered at 135 kg live weight (364 ± 58 days of age) due to their slow growth whilst crossbreeds were slaughtered at approximately 155 kg live weight (300 ± 30 days of age). These weights were chosen base on the commercial weights of both genetic types.

Pigs of the two genetic types were slaughtered in three days. At 40 min postmortem carcasses were eviscerated, weighted and chilled for 3 h at 2 °C. After the chilling, samples of the *longissimus thoracis* (LT) muscle at the level of $6^{th}/7^{th}$ thoracic vertebrae were collected and stored at -18 °C until further analysis.

2.2 Fatty acid analyses

FA composition was measured from *longissimus thoracis* samples and lipids were extracted according to Folch et al. (1957). A cold transmethylation was performed on fat according to Christopherson and Glass (1969), with some modifications, to convert fatty acids to the corresponding methyl esters (FAME). About 50 mg of fat was weighed in a conical vial and dissolved in 1 mL of n-hexane, added to 1 mL of the internal standard solution (2.1 mg of tridecanoic acid methyl ester dissolved in n-hexane) and 100 µL of KOH in methanol (c = 2 mol/L). The mixture was vigorously shaken for 30 sec and centrifuged at 252 x g for 3 min. 1 mL of the supernatant was diluted with 2 mL of n-hexane. 1 µL of the organic solution was analyzed by capillary gas chromatography (CGC) employing a RTX-2330 fused silica capillary column coated with 90% bis-cyanopropyl/10% phenyl cyanopropyl polysiloxane (30 m × 0.25 mm i.d., 0.2 µm f.t.) from Restek (Bellefonte, PA, USA) that was fitted on a Clarus 500 gas chromatograph from Perkin Elmer (Shelton, CT, USA). The injector and detector temperatures were set at 240°C. Helium was used as carrier gas at the flow of 1.25 mL/min. The oven temperature was held at 120°C for 1 min, increased from 120°C to 240°C at 4.0°C/min and finally held at 240°C for 10 min. The split ratio was set at 1:40. Peak identification was accomplished by comparing the retention times with those of two FAME standard mixtures: GLC-463 from Nu-Check (Elysian, MN, USA) and FAME 189-19 from Sigma (St. Louis, MO, USA). From each carcass sample were carried out three lipid extractions and the FAMEs composition was measured in 2 replicates for each lipid extract. FA were expressed as percentage on the total FA. Furthermore, as proposed by Boschetti et al. (2016), in addition to the omega 6 to omega 3 PUFA ratio (*n*-6/*n*-3), the following FA ratios were

estimated: the palmitoleic isomers to palmitic acid (C16:1/C16:0), the oleic to stearic acids (C18:1/C18:0), the dihomo-γ-linolenic to linoleic acid (C20:3 *n*-6/C18:2 *n*-6), the docosapentaenoic to adrenic acid (C22:5 *n*-3/C22:4 *n*-6) and the arachidonic to linoleic acid ratios (C20:4 *n*-6/C18:2 *n*-6).

2.3 Statistical analysis

Data were analysed using the mixed model procedure of SAS (SAS version 9.3. Cary, NC, USA), including the fixed effect of slaughter day, sex and genetic type, and the random effect of the subject within the day of slaughter. Means were compared using the Turkey-Kramer test, comparisons showing a $P< 0.05$ were considered significant and a trend towards significance was considered for those showing a $P< 0.10$.

3. Results and discussion

The levels of IMF noticed for Apulo-Calabrese and crossbreeds were within the range of values that meet consumers' acceptance (Fernandez et al., 1999). The effect of the genetic type on the fat content and FA profile in the LT muscle is shown in Table 1.

Table 1. Fatty acid composition of the *longissimus thoracis* muscle of 51 Apulo-Calabrese and 52 crossbreed pigs with the significance for the genetic type effect.

Traits	**Apulo-Calabrese**	**Crossbreeds**	***P*-value**
Intramuscular fat (IMF; g/100 g of muscle)	2.09 ± 0.14	1.68 ± 0.20	0.08
Fatty acids (FA; % on total FA)			
C10:0 (Capric acid)	0.10 ± 0.00	0.10 ± 0.00	ns
C12:0 (Lauric acid)	0.11 ± 0.00	0.11 ± 0.01	ns
C14:0 (Myristic acid)	1.40 ± 0.02	1.27 ± 0.03	0.03
C14:1 (Myristoleic acid)	0.02 ± 0.00	0.01 ± 0.00	0.004
C15:0 (Pentadecylic acid)	0.05 ± 0.00	0.04 ± 0.00	0.09

C16:0 (Palmitic acid)	24.15 ± 0.25	23.27 ± 0.44	ns
C16:1 *n*-9 (Cis-7 Hexadecenoic acid)	0.36 ± 0.02	0.34 ± 0.03	ns
C16:1 *n*-7 (Palmitoleic acid)	3.14 ± 0.07	2.78 ± 0.12	0.01
C17:0 (Margaric acid)	0.21 ± 0.00	0.18 ± 0.01	ns
C17:1 (Heptadecenoic acid)	0.20 ± 0.00	0.15 ± 0.00	<.0001
C18:0 (Stearic acid)	12.62 ± 0.24	12.81 ± 0.41	ns
C18:1 isomer (Octadecenoic acid isomer)	0.24 ± 0.29	0.53 ± 0.49	ns
C18:1 *n*-9 (Oleic acid)	38.24 ± 0.93	38.23 ± 1.59	ns
C18:1 *cis*-11 (Vaccenic acid)	5.74 ± 0.59	4.49 ± 1.00	ns
C18:2 *n*-6 (Linoleic acid)	8.72 ± 0.43	9.79 ± 0.74	ns
C18:3 *n*-6 (γ-linolenic acid)	0.07± 0.00	0.05± 0.01	ns
C18:3 *n*-3 (α-linolenic acid)	0.26 ± 0.01	0.28 ± 0.02	ns
C20:0 (Arachidic acid)	0.26 ± 0.01	0.15± 0.00	0.04
C20:1 (Gadoleic acid)	0.76 ± 0.00	0.69 ± 0.01	0.01
C20:2 *n*-6 (Eicosadienoic acid)	0.40 ± 0.014	0.43 ± 0.025	ns
C20:3 *n*-6 (Dihomo-γ-linolenic acid)	0.27 ± 0.02	0.29 ± 0.03	ns
C20:4 *n*-6 (Arachidonic acid)	1.76 ±0.18	2.76 ± 0.30	0.01
C20:3 *n*-3 (Eicosatrienoic acid)	0.12 ± 0.00	0.16 ± 0.02	0.06
C22:2 *n*-6 (Docosadienoic acid)	0.11 ± 0.00	0.12 ± 0.01	ns
C22:5 *n*-3/C22:4 *n*-6 (ratio of docosapentaenoic acid on adrenic acid)	0.32 ± 0.02	0.43 ± 0.04	0.01
C20:5 *n*-3 (Eicosapentaenoic acid)	0.09 ± 0.01	0.43 ± 0.04	ns
C24:0 (Lignoceric acid)	0.01 ± 0.00	0.01 ± 0.01	ns
C22:5 *n*-3 (Docosapentaenoic acid)	0.26 ± 0.02	0.30± 0.04	ns
C22:6 *n*-3 (Docosahexaenoic acid)	0.03 ± 0.00	0.08 ± 0.02	0.01
SFA (Saturated fatty acids)	38.82 ± 0.28	37.96 ± 0.49	ns
MUFA (Monounsaturated fatty acids)	48.76 ± 0.64	47.26 ± 1.09	ns
PUFA (Polyunsaturated fatty acids)	12.42 ± 0.67	14.77 ± 1.15	0.09
UFA (Unsaturated fatty acids)	61.18 ± 0.28	62.04 ± 0.49	ns
n-3 PUFA (omega 3 Polyunsaturated fatty acids)	0.77 ± 0.04	0.90 ± 0.07	0.08
n-6 PUFA (omega 6 Polyunsaturated fatty acids)	11.65 ± 0.64	13.88 ± 1.10	0.09
n-6/*n*-3 (ratio of omega 6 on omega 3 polyunsaturated fatty acids)	15.49 ± 0.47	15.97 ± 0.80	ns
C16:1/C16:0 (ratio of palmitoleic isomers and palmitic acid)	0.14 ± 0.00	0.13 ± 0.00	0.04
C18:1/C18:0 (ratio of oleic acid and stearic acid)	3.54 ± 0.07	3.38 ± 0.11	ns
C20:3 *n*-6/C18:2 *n*-6 (ratio of dihomo-γ-linolenic acid and linoleic acid)	0.03 ± 0.00	0.03 ± 0.00	ns
C20:4 *n*-6/C18:2 *n*-6 (ratio of arachidonic acid and linoleic acid)	0.20 ± 0.00	0.26 ± 0.00	0.03
MUFA/PUFA (ratio of monounsaturated fatty acids on polyunsaturated fatty acids)	4.57 ± 0.21	3.95 ± 0.36	ns
MUFA/SFA (ratio of monounsaturated fatty acids on saturated fatty acids)	1.26 ± 0.02	1.25 ± 0.03	ns
PUFA/SFA (ratio of polyunsaturated fatty acids on saturated fatty acids)	0.33 ± 0.02	0.40 ± 0.03	0.07

ns: not significant.

Local pig breeds have been reported to have a higher propensity for fat deposition, anyway in the present study the two genetic types showed only a trend towards significance (P= 0.08) for the IMF percentage in LT (Table 1). Concerning LT FA composition in both genetic types, the most predominant FA were oleic (C18:1 *n*-9), palmitic (C16:0), stearic (C18:0) and linoleic acids (C18:2 *n-6*). The most significant variation between the two genetic types was noticed for heptadecenoic acid, which is a minor constituent in monogastric animals' fat and its content in pork was already reported in Lo Fiego et al. (2010). No significant differences were found in the contents of total SFA and MUFA between the two genetic types. However, significantly higher contents of myristic (C14:0) and arachidic (C20:0) acids were found in Apulo-Calabrese. Similar findings were reported by Tomovic et al. (2016) for C14:0 when White Mangalica pigs were compared with crossbreeds and Large White pigs but showed the opposite trend for C20:0. According to literature, autochthonous breeds are a rich source of MUFA, which was confirmed by the significantly higher contents of myristoleic (C14:1), palmitoleic (C16:1 *n*-7), heptadecenoic (C17:1), gondoic (C20:1) acids and palmitoleic isomers to palmitic acid ratio (C16:1/C16:0) in the meat of Apulo-Calabrese compared with crossbred pigs (Table 1). The samples of Apulo-Calabrese pigs showed significantly lower contents of docosahexaenoic (C22:6 *n-3*) and arachidonic (C20:4 *n*-6) acids, and a tendency to lower contents of eicosatrienoic (20:3 *n*-3), PUFA, *n*-3 PUFA, *n*-6 PUFA and the PUFA/SFA ratio (Table 1). Similarly, Nevrkla et al. (2017) observed significantly lower contents of eicosatrienoic, arachidonic and docosahexaenoic acids in Prestice Black-Pied pigs than in hybrid pigs. The difference in PUFA content between crossbreed and Italian local pigs may be due to the different growth performance and adipogenic potential characterising the different genetic types considered. On the whole, these results are in agreement with the fact that lower is the amount of fat stored, the higher is the proportion of PUFA on the total FA (Wood et al., 2008). The reason for this is that PUFA are essential components of cell membranes, and while the storage of energy through SFA may change among individuals and over time, the amount of PUFA remains stable due to their important roles in membranes flexibility. Interestingly, despite the consideration of Apulo-

Calabrese as a breed with a high adipogenic potential, the present study did not find great differences between the crossbreed samples and those belonging to this local breed, in agreement with the results on meat quality traits reported in our previous study (Aboagye et al., 2018).

4. Conclusions

The results from this study indicated that when Apulo-Calabrese pigs are reared in the indoor system and fed the same commercial diet as crossbreeds, their *longissimus thoracis* muscle fatty acid composition is similar to those observed in commercial crossbreed pigs. However, further research is needed to better differentiate and to improve the knowledge of the biological mechanisms underlying the fatty acid profile of local and commercial pig breeds.

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Conflict of Interest Statement

The authors warrant that there are no any conflicts of interests, financial or personal, among authors, between authors, other people, and institutions or organizations.

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Supplementary material

Supplementary Table 1. The composition of the finishing diet fed to Apulo-Calabrese and Crossbreed pigs.

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