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EFFECT OF DIETARY ANTIOXIDANT SUPPLEMENTATION ON RABBIT PERFORMANCE, MEAT QUALITY AND OXIDATIVE STABILITY OF MUSCLES

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Abstract: The aim of this study was to cast light on the effects of EconomasE™ (EcoE), a patented pre-mixture of nutritional additives consisting mainly of organic selenium (0.15 or 0.30 mg/kg feed; Se) combined with vitamin C (5 and 10 mg/kg feed; VC), compared to DL-α-tocopherol acetate (100 or 200 mg/kg feed; VE) dietary supplementation on rabbit performance and meat quality. In fact, the role of Se supplementation in the rabbit diet has not yet been elucidated in the literature and, more specifically, there are no studies on the possible synergistic action between organic Se compared with VE on lipids, fatty acids (FA) and the oxidative stability of two glycolytic muscles, *longissimus lumborum* (LL) and *biceps femoris* (BF). Two hundred and seventy New Zealand White rabbits were divided into five dietary groups of 54 rabbits each: 1) control (basal diet=BD; CTRL); 2) VE100 (BD+VE100 mg/kg); 3) VE200 (BD+VE200 mg/kg); 4) EcoE100 (BD+EcoE100 mg/kg); and 5) EcoE200 (BD+EcoE200 mg/kg). Neither of the antioxidant treatments affected growth performance, carcass traits or meat characteristics. Lipid and fatty acid contents were similar in LL and BF and not influenced by the dietary treatment. Meat oxidative stability was strongly improved by both antioxidants. These findings indicate that both EcoE and VE greatly improved the oxidative stability of LL and BF muscles at the dosage rates which, from an economic point of view, would normally be included in the formulation of feeds for rabbits.

Key Words: rabbit, EconomasE™, selenium, vitamin E, oxidative stability, meat quality.

INTRODUCTION

Rabbit meat consumption is ranked fourth in Italy, with over 90% self-sufficiency (Dalle Zotte and Szendrő, 2011). The data highlight that global rabbit meat production has steadily increased during the period of 2010-2016 and that Europe, as a whole the second largest rabbit meat producing region, currently accounts for almost 93% and 67% of the world's rabbit meat imports and exports, respectively (Cullere and Dalle Zotte, 2018). Compared to other meats, rabbit meat has high nutritional value as a result of its lipid component, characterised by a comparatively low fat and cholesterol level, higher unsaturated fatty acids (UFA) and the best ratio of n-6/n-3 polyunsaturated fatty acids (PUFA) (Dalle Zotte and Szendrő, 2011; Montero-Vicente *et al.,* 2018; Petrescu and Petrescu-Mag, 2018). However, the higher amount of UFA leads to a decrease in product stability during storage and cooking. Indeed, lipid oxidation is one of the main non-microbial factors responsible for the deterioration of rabbit meat quality, with an ensuing reduction of shelf-life (Kouba *et al.,* 2008).

In livestock production, several nutritional strategies have been devised to delay quality deterioration (Xiao *et al.,* 2011; Albonetti *et al.,* 2017). For example, to prevent lipid oxidation, the use of antioxidants such as vitamin E (VE)

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(Lo Fiego *et al.,* 2004; Corino *et al.,* 2007; Eiben *et al.,* 2011) or Se (Dokoupilová *et al.,* 2007; Matics *et al.,* 2017; Papadomichelakis *et al.,* 2017) has been tested. VE dietary supplementation increases its content in meat with an ensuing better oxidative stability in the raw and cooked state (Castellini *et al.,* 2001). While the role of VE in stabilising meat colour and reducing oxidative processes has been assessed (Dalle Zotte and Szendrő, 2011), the antioxidant role of Se is still not clear (Abdel-Khalek *et al.,* 2013; Papadomichelakis *et al.,* 2017) and the effects of Se, either organic or inorganic, as dietary supplementation on its final concentration in rabbit meat and on meat quality are not conclusive. Indeed, even if there is no clear recommendation as to the appropriate amount of supplemental Se in the feeds of rabbits, the minimum Se requirements for a normal growth are quite low (0.08 mg/kg of feed), and yet small amounts of Se are advisable, because of its potential effects on other physiological functions (Dokoupilová *et al.,* 2007; Mateos *et al.,* 2010; Papadomichelakis *et al.,* 2017).

The addition of 0.12 to 0.50 mg/kg of Se in feed increases its content in muscle (Mateos *et al.,* 2010); at higher levels than those which are strictly necessary (0.1 mg/kg feed), it does not affect either glutathione peroxidase (GPx) activity or the oxidative stability of rabbit meat (Dokoupilová *et al.,* 2007). As for other species (Xiao *et al.,* 2011; Nambapana *et al.,* 2015; Chauhan *et al.,* 2016), the administration of a source of UFAs associated with an antioxidant substance of algal origin (EconomasE^{TM}, EcoE) containing Se and combined with vitamin C (VC) might have a positive synergistic effect on oxidative stability in rabbit and be more cost-effective than the VE alone, given the VE-saving activity of EcoE.

The use of antioxidants during rabbit fattening could have a protective effect against microbial contamination (Albonetti *et al.,* 2017), as well as the susceptibility to feed mycotoxin contamination, which may stimulate lipid peroxidation (Mézes and Balogh, 2009). A fine balance between antioxidants and pro-oxidants regulates the metabolic pathways under stress conditions and strongly influences the animal's commercial production. Antioxidants may adjust that balance, while nutritional stressors may have a negative impact.

In this work, the effect of EcoE, a patented pre-mixture of nutritional additives consisting mainly of Se and VC, on rabbit zootechnical parameters of growth and meat quality is compared with that of VE. In addition, the effects of the antioxidants on lipid content, FA composition and the oxidative stability of predominantly glycolytic *longissimus lumborum* (LL) and *biceps femoris* (BF) muscles are evaluated.

MATERIALS AND METHODS

Animals and diets

The experimental protocol was approved by the Scientific Ethics Committee on Animal Experimentation (University of Bologna, Prot. ID 1/72/2012). A total of 270 thirty-five days old weaned New Zealand White male rabbits were randomly divided into five dietary groups of 54 rabbits each; these were subdivided into three collective cages (3 replicates; 18 animals/cage, 0.084 m^2/head), with *ad libitum* access to feed and water: 1) control (basal diet=BD; CTRL); 2) VE100 (BD+VE 100 mg/kg); 3) VE200 (BD+VE 200 mg/kg); 4) EcoE100 (BD+100 mg/kg of EcoE (Alltech, Nicholasville, KY, USA); and 5) EcoE200 (BD+EcoE 200 mg/kg). The organic Se produced by *Saccharomyces cerevisiae* CNCMI-3060 in the premix (750000 mg/kg) corresponds to an Se amount of 1500 mg/kg feed. The 100 and 200 mg/kg added to the EcoE100 and EcoE200 diets contained 0.15 mg and 0.30 mg/kg of Se, respectively. In the EcoE premix, the VC amount (50000 mg/kg) corresponded to 5 and 10 mg/kg feed in EcoE100 and EcoE200 diets, respectively.

Both a starter basal diet (SBD) and a finisher basal diet (FBD) (Table 1), respectively used in the first (5-9 wk) and in the second period (9-12 wk), were supplemented with 50 mg/kg of VE (Sigma-Aldrich, St. Louis, MO, USA) and 0.22 mg/kg of Na_2SeO_3 corresponding to an Se amount of 0.099 mg/kg feed.

Growth performance

Live body weight (BW) was determined at 5, 9 (change of diet) and 12 weeks of age and feed intake (FI) was measured on a cage basis twice a week. Average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR) and mortality (daily recorded) were calculated for each period, on three cages (18 rabbits/cage) per dietary group (approximately 54 rabbits/d.g.).

Slaughter traits and muscle sampling

Rabbits were transported to the slaughterhouse at 85 d of age, weighted and electrically stunned (70 V DC, 50 Hz, 5 s). The slaughtering and carcass dissection procedures were carried out according to Blasco and Ouhayoun (1993). After complete bleeding, skin and viscera were removed and the hot carcasses (HC) were weighed and chilled at 4°C for 24 h in a refrigerated cell (Costan Daily TN SP60/4, Costan S.p.A., Belluno, Italy).

Ten carcasses were randomly selected from each dietary group for qualitative analysis. Each chilled carcass (CC) was weighed, and the head, thymus, trachea, oesophagus, heart, lungs, liver and kidneys were removed to obtain the reference carcass (RC) (Blasco and Ouhayoun, 1993). The ratio of head, liver and carcass parts to either the CC or RC were calculated as required. The BF, *longissimus thoracis* (LT) and LL left muscles were dissected at 24 h *post-mortem* (Blasco and Ouhayoun, 1993), carefully freed from connective and adipose tissues, and used to determine the meat quality parameters. The LL and BF right muscles were stored in liquid nitrogen until FA and lipid oxidation analysis.

Feed chemical composition

The chemical composition of feed was determined (AOAC, 2000) for dry matter (934.01), crude protein (2001.11), crude fibre (978.10), ash (967.05) and starch (996.11). Ether extract was determined after acid-hydrolysis (AOAC, 2006, 963.15). Neutral detergent fibre (NDF, without sodium sulphite), acid detergent fibre and acid detergent lignin were determined according to Van Soest *et al.* (1991), where NDF was assayed with a heat stable amylase and expressed inclusive of residual ash. Crude fibre was determined only to enter the corresponding value in the feed manufacturer sheet, as required by law. Calcium analysis was performed by Atomic Absorption Spectrometry (Thermo Solaar S4-Local Controller) according to Martillotti *et al.* (1987). Phosphorus was determined by spectrophotometric method (AOAC, 2000, 991.27). Gross energy (MJ/kg) was measured with an adiabatic bomb calorimeter (ISO, 1998). Feed analyses were performed in triplicate. The SBD and FBD chemical compositions used in the five dietary groups are shown in Table 1.

Lipid and FA content in SBD and FBD used in the five dietary groups are shown in Tables 2 and 3, respectively.

Table 1: Ingredients and chemical composition of the starter and finisher rabbit basal diet.

	Basal diets	
	SBD	FBD
Ingredients (g/kg diet)		
Wheat bran	250	160
Sunflower seed	195	215
Sugar beet pulp	150	150
Alfalfa hay	100	90
Barley	NP	85
Alfalfa dehydrated	80	30
Wheat flour middlings	50	70
Sugarcane molasses	50	50
Grape seeds meal	NP	50
Oats	40	40
Olive-pomace oil	35	NP
Calcium carbonate	12	8
Soybean mill run	NP	20
Palm oil	8	11
Sodium chloride	4	4
Soybean oil	3.3	3.3
Supplement	22.7	13.7
Vitamin E	0.05	0.05
Selenium (mg/kg)	0.1	0.1
Chemical composition (g/kg as feed)[1]		
Dry matter	885.1±1.2	890.5±0.6
Crude protein	164.3±0.9	164.1±0.9
Ether extract	42.6±0.5	47.5±0.4
Crude fibre	194.5±4.4	160.5±1.9
Starch	153.0±5.3	228.4±6.7
Ash	105.9±1.0	81.5±0.4
Neutral detergent fibre	411.4±5.2	329.8±2.9
Acid detergent fibre	271.7±5.1	209.6±1.7
Acid detergent lignin	71.7±2.1	69.3±3.1
Ca	17.8±0.5	13.4±0.2
P	5.5±0.1	6.5±0.1
Gross energy (MJ/kg)	16.1±0.2	16.7±0.2

SBD: starter basal diet used in the first period (5-9 wk); FBD: finisher basal diet used in the second period (9-12 wk); NP: not present.
[1]n=3.

Table 2: Fatty acid (FA) content of the starter rabbit basal diet.

FA content[1]	Dietary groups				
(% of total FA)	CTRL	VE100	VE200	EcoE100	EcoE200
C13:0	1.21±0.13	1.14±0.08	0.94±0.06	0.96±0.14	0.84±0.06
C14:0	0.37±0.01	0.30±0.01	0.31±0.04	0.38±0.01	0.32±0.03
C14:1n-5	0.08±0.01	0.08±0.01	ND	ND	ND
C15:0	0.10±0.01	0.09±0.00	0.09±0.01	0.12±0.02	0.08±0.01
C16:0	20.75±1.01	20.03±0.84	19.52±0.74	22.51±0.03	20.96±0.65
C16:1n-7	0.22±0.02	0.21±0.01	0.25±0.00	0.22±0.01	0.23±0.01
C17:0	0.14±0.02	0.11±0.00	0.11±0.00	0.13±0.00	0.14±0.02
C17:1n-7	0.08±0.01	0.06±0.00	0.08±0.01	ND	0.10±0.04
C18:0	3.08±0.02	3.00±0.08	3.14±0.01	3.44±0.06	3.26±0.02
C18:1n-9	29.22±0.14	29.46±0.14	29.89±0.22	29.08±0.25	29.08±0.38
C18:1n-7	0.15±0.00	0.24±0.01	0.22±0.01	0.33±0.00	0.20±0.06
C18:2n-6	39.46±0.71	40.20±0.64	40.07±0.45	37.32±0.04	39.42±0.44
C18:3n-3	3.58±0.02	3.65±0.08	3.58±0.08	3.16±0.02	3.38±0.01
C20:0	ND	ND	0.28±0.01	0.29±0.01	0.28±0.02
C20:1n-9	0.45±0.01	0.46±0.01	0.46±0.01	0.41±0.00	0.41±0.02
C20:2n-6	0.07±0.01	0.06±0.00	0.08±0.01	ND	ND
C20:4n-3	0.46±0.01	0.44±0.02	0.49±0.03	0.49±0.04	0.42±0.04
C20:5n-3	0.15±0.04	0.14±0.02	0.15±0.04	0.15±0.03	0.11±0.02
C22:4n-6	0.12±0.00	0.14±0.01	0.14±0.01	0.13±0.01	0.12±0.01
C22:6n-3	0.31±0.04	0.20±0.02	0.18±0.02	0.90±0.02	0.63±0.02

[1]n=2, mean±standard error. Lack of letters within the same row indicate absence of significant differences at $P<0.05$.
CTRL: Control diet (SBD: starter basal diet used in the first period, 5-9 wk); VE100: SBD supplemented with 100 mg of Vitamin E per kg of feed; VE200: SBD supplemented with 200 mg of Vitamin E per kg of feed; EcoE100: SBD supplemented with 100 mg of EconomasE™ per kg of feed; EcoE200: SBD supplemented with 200 mg of EconomasE™ per kg of feed. ND: not detectable.

Mycotoxin detection

As this study was included in a standard industrial process, to exclude the presence of such substances, for hygienic and sanitary purposes the mycotoxin analysis was performed both on SBD and FBD feeds and on livers using an enzyme immunoassay for the detection of aflatoxin B1 (AFB1, MA220/MA221, Tecna, Italy) and ochratoxin A (OTA, *I'screen* OR361, Tecna, Italy). Mycological analyses were carried out on both feeds to detect the presence of moulds on Yeast Extract Sucrose agar plates.

α-Tocopherol (αT) content

The αT content was determined in feeds and minced meat. Feed samples (500 mg) were homogenised, spiked with 20 µL of αT internal standard stock solution (IS, 100 mg/kg) and then extracted with 2 mL of petroleum ether without a saponification process. Minced meat samples (100 mg), thawed and homogenised by an Ultra-Turrax, were spiked with 50 µL of IS working solution (1 mg/kg) and extracted with a saponification process. Filtered supernatant was injected into the liquid chromatography - tandem mass spectrometry system. The αT was determined using an Acquity UPLC (Waters Corporation, Milford, MA, USA). A Waters BEH C18 column with a guard column was used for the isocratic separation with 100% MeOH and 2.5 mM CH_5NO_2 as a mobile phase (Midttun and Ueland, 2011). A Quattro Premiere XE with an ESCI™ Multi-Mode Ionisation Source (Waters) was used for the mass spectrometric analysis. Data acquisition processing was performed using Mass Lynx 4.1 Software (Waters).

Table 3: Fatty acid (FA) content of the finisher rabbit basal diet.

FA content[1]	Dietary groups				
(% of total FA)	CTRL	VE100	VE200	EcoE100	EcoE200
C13:0	1.52±0.10	2.04±0.44	0.86±0.35	1.22±0.18	1.06±0.26
C14:0	0.50±0.04	0.56±0.01	0.47±0.00	0.52±0.02	0.50±0.01
C14:1n-5	ND	ND	ND	ND	ND
C15:0	0.12±0.01	0.14±0.02	0.12±0.00	0.12±0.01	0.10±0.01
C16:0	17.38±0.44	19.54±0.64	17.60±0.34	20.07±0.10	18.77±0.17
C16:1n-7	0.45±0.02	0.54±0.04	0.46±0.02	0.50±0.01	0.44±0.01
C17:0	0.26±0.00	0.24±0.05	0.21±0.00	0.26±0.02	0.22±0.01
C17:1n-7	0.24±0.04	0.12±0.01	0.23±0.01	0.22±0.05	0.12±0.00
C18:0	4.98±0.02	4.84±0.04	5.27±0.07	5.61±0.15	5.71±0.24
C18:1n-9	27.18±0.10	26.03±0.50	27.18±0.89	26.50±0.66	27.16±0.56
C18:1n-7	0.47±0.04	0.52±0.01	0.43±0.06	0.46±0.06	0.39±0.14
C18:2n-6	42.17±0.30	40.98±0.78	43.06±0.16	40.20±0.33	41.45±0.31
C18:3n-3	2.60±0.06	2.76±0.12	2.73±0.12	2.48±0.09	2.69±0.17
C20:0	0.32±0.01	0.33±0.08	0.30±0.01	0.30±0.01	0.26±0.00
C20:1n-9	0.48±0.04	0.50±0.12	0.42±0.04	0.40±0.02	0.46±0.07
C20:2n-6	ND	ND	ND	ND	ND
C20:4n-3	0.46±0.04	0.61±0.01	0.49±0.02	0.42±0.08	0.44±0.06
C20:5n-3	0.34±0.01	0.12±0.01	ND	0.14±0.01	0.08±0.00
C22:4n-6	0.32±0.01	0.09±0.00	ND	ND	ND
C22:6n-3	0.20±0.01	0.06±0.01	0.18±0.01	0.58±0.10	0.40±0.08

[1]n=2, mean±standard error. Lack of letters within the same row indicate absence of significant differences at $P<0.05$.

CTRL: Control diet (FBD: finisher basal diet used in the second period, 9-12 wk); VE100: FBD supplemented with 100 mg of Vitamin E per kg of feed; VE200: FBD supplemented with 200 mg of Vitamin E per kg of feed; EcoE100: FBD supplemented with 100 mg of EconomasE™ per kg of feed; EcoE200: FBD supplemented with 200 mg of EconomasE™ per kg of feed. ND: not detectable.

Se content

Feed (0.5 g) or minced meat (1 g) samples were mineralised with 6 mL of 67% HNO_3 in a microwave digestion system Milestone Ethos One following US-EPA method 3052 (U.S. EPA, 1986). Se was determined using an Optima 2100 DV (PerkinElmer) ICP-OES following US-EPA method 6010c (U.S. EPA, 1986) combined with hydride generation technique to reduce interference. At the wavelength of 196.026, Se was read with the axial position of the torch. The calibration line was produced passing through zero, with the correlation coefficient equal to 0.999972.

Meat quality evaluation

The ultimate pH (pHu) was measured in the left LL muscle at the 5th-6th lumbar vertebrae level using a pH-metre (HI92240, Hanna Instruments, Padova, Italy) with a penetration electrode (Double-Pore cod. n° 32384003, Hamilton).

The colour parameters (L^*, a^*, b^*) of the left LL muscle were evaluated using a tristimulus analyser (Minolta Chroma-Meter CR-200, Minolta Inc., Osaka, Japan) according to the CIE LAB (CIE, 1976); from the chromaticity coordinates, hue and chroma were calculated (McLellan *et al.,* 1995).

The cooking losses of the whole dissected LT, LL and BF muscles were determined.

To determine cooking loss, the whole dissected LT, LL and BF muscles from the left side of each carcass were individually weighed and recorded as initial weight (W1). The weighted samples (LT, LL and BF approximately: 18, 75 and 38 g, respectively) were individually vacuum-sealed in PVC bags and cooked for 10 min in a pre-heated water bath set at 80°C to ensure a core temperature of 77°C, detected by a thermometer equipped with a Type J (iron-

constantan) thermocouple (EUROTRON, Micrologger 2, Sesto San Giovanni, Italy). The cooked samples were then removed from the water bath, equilibrated at room temperature, removed from the bag, blotted dry using paper towels without any squeezing and reweighed (W2). The cooking loss percentage was calculated according to Honikel (1998) using the following equation: Cooking loss (%)=[(W1–W2)÷W1]×100.

Cooked left LT, LL and BF muscles were then used for the shear force (WBSF) determination on one (for LT, BF) or three (for LL) cylindrical cores (diameter 1.25 cm) sheared perpendicularly to the muscle fibre direction with a Warner-Brätzler meat shear device fitted on an INSTRON 1011 (Instron Corp., Italy) machine (AMSA, 1995). WBSF was expressed as the peak corresponding to the maximum shear force (N/cm^2).

After cooking, meat colour was measured four times on the left LL muscle (approximately 60 g) following the method previously described.

Raw and cooked LL proximate composition

Meat samples of right LL muscle were minced using a blender homogeniser and frozen at –20°C. The samples were lyophilised and then dried at 105°C until constant weight (Giaretta *et al.,* 2019). Ground freeze-dried samples were analysed (AOAC, 2006) for moisture (950.46), crude protein (981.10) and ash (920.153). Crude protein was calculated using a Kjeldahl-Nitrogen/Protein-Analyser (Gerhardt Vapodest 50, Gerhardt GMBH, Italy). Fat content was determined by the Soxhlet method (2055 Soxtec Avanti, Foss Tecator AB, Höganäs, Sweden) (991.36; AOAC, 2006). The energy value (kJ/100 g lean) was calculated multiplying the protein and fat amount by conversion factor 17 and 37, respectively (EC, 2011).

Lipid extraction and FA analysis

Analyses were carried out on basal diets (SBD and FBD) and on LL and BF right muscles. Total lipids were extracted according to Bligh and Dyer (1959) and directly transmethylated (Ichihara *et al.,* 1996). Fatty acid methyl esters (FAME) were analysed on a Varian 3380 gas chromatograph according to Pirini *et al.* (2007). Data were processed using a Varian Star Chromatography Workstation and each peak was identified with pure FAME standard mixtures. The content of fatty acids was expressed as % of total FA.

The amount of each FA was used to calculate the sum of the saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) and the indexes of atherogenicity (AI) and thrombogenicity (TI) (Ulbricht and Southgate, 1991), as well as that of peroxidability (PI; Erickson, 1992).

Lipid oxidation

Lipid oxidation in the right LL and BF was spectrophotometrically evaluated through the iron-induced thiobarbituric acid-reactive substances (TBARS) (Maraschiello *et al.,* 1999) slightly modified using 2 g of tissue homogenised with an Ultra-Turrax in 18 mL of 40 mmol Tris-maleate buffer (pH 7.4) with 1 mmol $FeSO_4$ to catalyse lipid peroxidation. After the addition of 5 mL of cold 25% trichloroacetic acid, each homogenate was placed for 30 min at 25°C and centrifuged at 13000×*g* for 15 min at 4°C. For each sample, 2.1 mL supernatant were transferred to screw-capped test tube and after the addition of 0.9 mL of 0.6% aqueous thiobarbituric acid, the samples were incubated 30 min at 70°C, cooled and their absorbance (532 nm) was recorded. The mg of malonaldehyde (MDA) per kg of sample were calculated based on a calibration curve using 1,1,3,3-tetraethoxypropane (TEP). In this study, the single 30 min-incubation time with $FeSO_4$ was chosen because, from various preliminary analyses at different times, it provided the most appropriate and reliable result both at the level of MDA quantification and of reproducibility, as also reported by Peiretti *et al.* (2011).

Statistical analysis

The rabbit dietary treatment was used as the source of variation to determine any significant differences. The individual animal was the experimental unit for the measured variables, except for zootechnical parameters. In this case, each cage was the experimental unit (three replicates/dietary treatment). The zootechnical data were firstly analysed by a two-way ANOVA for repeated measures, using Tukey's HSD as *post-hoc* test to verify: i) the dietary effect and ii) the

cage effect (as fixed effect). Since the cage effect was not significant, it was not reported and/or discussed in the text. Mortality data were subjected to statistical analysis using the χ^2 test.

Data on lipid analyses and TBARS were presented as mean values (n=10). To compare the differences between dietary treatments and control, and between muscles, all data were submitted to a two-way ANOVA with diet and muscle as fixed effects followed by the Student-Newman-Keuls test. Statistically significant differences are shown in the tables. Before statistical analyses, data were checked for variance homogeneity and normal distribution and, when required, to ensure normality percentage data were transformed (arcsin square root).

Data on VE and Se contents, reported as mg/kg of feed or meat, were statistically analysed by a one-way ANOVA, followed by the Student-Newman-Keuls test.

All statistical analyses were performed using SigmaStat® release 2.0 (SPSS Inc. Chicago, USA) with a significance level of $P<0.05$.

RESULTS AND DISCUSSION

Productive performance and carcass traits

The zootechnical performance was in accordance with those of rabbits in trading conditions (Cullere and Dalle Zotte, 2018; EFSA AHAW Panel, 2020). As for live weights at slaughter (Table 4), no significant differences were observed between groups.

Table 4: Effect of dietary supplementation with VE or EcoE on growth performance from 5 to 12 wk of age and on carcass traits.

	Dietary groups						
	CTRL	VE100	VE200	EcoE100	EcoE200	SEM	*P*-value
Growth performance							
Nº of rabbits	53	52	51	47	53		
Initial BW (g)	922.6	923.0	914.2	921.3	922.9	14.3	0.988
Final BW (g)	2991	2934	2867	2905	2981	34	0.058
ADG (g/d)	41.38[b]	40.21[ab]	39.06[a]	39.67[ab]	41.15[ab]	0.58	0.023
ADFI (g/d)	175.3	166.7	168.8	165.4	170.8	2.4	0.711
FCR (g feed/g gain)	4.07	4.06	4.11	3.94	3.99	0.12	0.845
Carcass traits							
Nº of rabbits	10	10	10	10	10		
CC (g)	1843	1793	1742	1841	1866	35.48	0.120
RC (g)	1530[ab]	1486[ab]	1449[a]	1560[b]	1543[ab]	27.01	0.039
RC yield, % CC	83.0	82.9	83.2	82.9	82.7	0.34	0.918
Ratio to CC in %:							
Head	8.94	9.35	9.27	9.21	9.20	0.22	0.738
Liver	5.25	4.88	4.80	5.27	5.17	0.21	0.407
Kidney	0.97	0.99	1.02	0.99	1.01	0.03	0.722
Ratio to RC in %:							
Loin	35.21	35.10	34.51	33.86	34.55	0.39	0.138
Hind leg	32.83	33.21	33.89	32.88	33.07	0.35	0.242
Perirenal fat	3.02	2.94	2.48	3.39	3.35	0.23	0.062

BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; CC: chilled carcass; RC: reference carcass. CTRL: Control diet (BD: basal diet); VE100: BD supplemented with 100 mg of Vitamin E per kg of feed; VE200: BD supplemented with 200 mg of Vitamin E per kg of feed; EcoE100: BD supplemented with 100 mg of EconomasE™ per kg of feed; EcoE200: BD supplemented with 200 mg of EconomasE™ per kg of feed. SEM: standard error of mean.
[ab]Different letters within the same row indicate significant differences ($P<0.05$).

The overall rabbit mortality (14 rabbits corresponding to 5.2% of the rabbit initial number) was low throughout the study period, irrespective of the dietary treatments. In the first growing period, mortality was very low and ranged from 5 (1.85% CTRL and 3.70% for each EcoE groups) to 9 (1.85% for each VE group) dead rabbits, whereas in the second growing period no deaths occurred.

The values of BW, ADFI and FCR in rabbits (Table 4) were not affected by dietary treatments. The ADG of the CTRL group was greater than that of the VE200 group, whereas the other dietary groups presented intermediate values.

As for the carcass traits (Table 4), only the RC weight of the VE200 group was statistically lower (–7.1%) than that of the EcoE100 group. No differences were found among the other treatments.

In growing rabbits, the lack of significant effect on enhancing growth performance due to supplemental antioxidants has been previously reported (Oriani *et al.,* 2001; Corino *et al.,* 2007; Dokoupilová *et al.,* 2007; Eiben *et al.,* 2011; Matics *et al.,* 2017), even though the experimental conditions were not exactly the same. In rabbits 61 d old fed for the last 4 wk on a diet supplemented with α-tocopheryl acetate (60, 150 or 375 mg/kg), there was no effect on rabbit growth (Oriani *et al.,* 2001). Likewise, growing rabbits fed with VE-supplemented diet (150 mg/kg) during the last 7 wk of fattening period had identical performance compared to those fed with a low VE level (60 mg/kg; Eiben *et al.,* 2011). Corino *et al.* (2007) found that in rabbits fifty-five days old a dietary supplementation with VE (240 mg/kg) for the last 5 wk was associated with a slight increase in BW at slaughter only in male rabbits compared to those fed with a low VE level (60 mg/kg). As for Se supplementation, Dokoupilová *et al.* (2007) reported that in rabbits 35 d old the dietary supplementation with Se-yeast (Sel-Plex®) at two different diet concentrations (0.12 and 0.50 mg/kg) for the last 4 wk did not affect growth, feed conversion and dressing out percentage. No effect on performance was found also in rabbits fed with Se-supplemented diet (Sel-Plex®, Se-algae or Sodium Selenite at the concentration of 0.40 mg Se/kg) compared to those fed with a basal diet containing 0.08 mg Se/kg (Marounek *et al.,* 2009). Similarly, productive traits were also not affected by Se 0.46 mg/kg added to the diet of growing rabbits for the last 7 wk (Matics *et al.,* 2017). Conversely, Ebeid *et al.* (2013) evidenced that in male rabbits at forty-two days of age fed for the last 6 wk with basal diet containing VE (6 mg/kg) and Se (0.03 mg Sel-Plex®/kg) supplemented with VE (250 mg/kg) or Se (0.3 mg Sel-Plex®/kg), both VE and Se had a positive effect on growth performance. However, Ebeid *et al.* (2013) noticed that the differences between their results and those highlighted in the literature (Dokoupilová *et al.,* 2007; Marounek *et al.,* 2009) could be related to differences between strains and in the housing systems.

The data on the carcass traits are consistent with Castellini *et al.* (2001), who did not find any difference in the carcass traits of rabbits fed with diet supplemented with VE (50 or 200 mg/kg) and VC (500 or 1000 mg/l^{-1} water), singly and in combination supplemented diets, during the entire fattening period (from 35 to 85 d). Other studies showed that those variables were unaffected by VE or Se supplementation (Dokoupilová *et al.,* 2007; Marounek *et al.,* 2009; Matics *et al.,* 2017; Oriani *et al.,* 2001), while Eiben *et al.* (2011) reported that rabbit carcass traits improved with the dietary supplementation of linseed oil and Se. Similarly, Ebeid *et al.* (2013) reported that VE and Se increased the rabbit HC weights, corresponding to their higher live BW, even though they did not influence the proportions of various carcass organs, as also reported by Eiben *et al.* (2011).

In conclusion, the previous literature as well as our results show that it is quite complex to compare data on the effects of VE and Se supplementation on productive and carcass traits, probably also due to differences in the housing systems of the rabbits. However, the growth results obtained in this study are in accordance with the breed parameters of the hybrid used.

Mycotoxin detection

The values of AFB1 and OTA in both feeds and liver samples were below the detection limit (<1 ng/g AFB1 in feed and liver; <1 ng/g and <0.1 ng/g for OTA in feed and liver, respectively. Therefore, high resolution analytical methods (ultra-performance liquid chromatography or liquid chromatography–mass spectrometry) were not required.

From the mycological analysis of the feeds, fungi of *Rhizopus* and *Aphanomyces* genera were purified and identified, whereas mycotoxigenic fungi of *Aspergillus* and *Fusarium* genera were not present.

α-Tocopherol content in feeds and rabbit meat

The αT content in feed and minced meat determined in the five dietary groups is shown in Table 5. The αT concentrations in feeds were significantly higher in the VE groups, as expected. In the CTRL and EcoE groups, the total αT values, not significantly different, were higher than expected (50 mg/kg as in the basal diet), while in the VE100 group it was slightly lower than expected (i.e. 150 mg/kg). In the VE200 group, the αT content corresponded to the supplemented dose, roughly three times higher than in the CTRL group.

In the meat samples, the αT levels were significantly higher in the VE groups than in the CTRL group (Table 5), but with no further increase in the VE concentration in the meat as the VE concentration in the basal diets increased. The αT levels in the VE groups were approximately two times higher than in the CTRL group. In the meat, the increase in αT concentration seems to depend on the dietary VE increase (Castellini *et al.,* 2001; Lo Fiego *et al.,* 2004), even if such an increase reported in the literature is not linear, especially at high VE concentrations (Abdel-Khalek, 2013; Lopez-Bote *et al.,* 1997; Oriani *et al.,* 2001). Moreover, it is possible that the αT variation does not physiologically allow for a saturation effect as a result of factors limiting VE transport or absorption in rabbits (Oriani *et al.,* 2001), the efficiency of which is inversely related to the quantity supplied. As expected, in the EcoE groups, the αT content was equal to that of the CTRL group.

Se content in feeds and rabbit meat

In feed (Table 5), the Se content of the five dietary groups corresponded to the Se supplied.

In meat samples (Table 5), Se concentration was significantly higher in the EcoE groups (+14%) than in the CTRL and VE groups, where the Se concentration was about the 0.1 mg/kg level usually recommended to cover the Se dietary requirement of rabbit (Mateos *et al.,* 2010). However, no significant differences were detected between EcoE dietary groups, indicating that there was no Se dose-dependent effect. Hence, in this study, the Se doses used for EcoE diets (i.e. 0.25 and 0.4 mg/kg feed in EcoE100 and EcoE200 groups, respectively) are to be considered supranutritional, yet still compatible with animal health, while Se at high doses, such as 2.5 mg/kg feed with even pro-oxidant effect (Papadomichelakis *et al.,* 2017), might lead to health risks for rabbits and, in perspective, it is not feasible for practical application because it is far too expensive.

Literature data for Se concentration in rabbit tissue are sparse, but a relatively small difference (16.5%) in the liver Se concentration was reported by Erdèlyi *et al.* (2000) in rabbits fed diets equivalent to that of our EcoE200 group. As the supranutritional Se seems not to cause drastic changes in Se concentration in the blood and liver, as well as in GPx activity, it is hypothesised that rabbits have an appropriate mechanism to get rid of excess Se, considering that slightly less than 50% of the Se intake in rabbits is excreted in the urine and faeces (Erdèlyi *et al.,* 2000). On the contrary, according to other research groups, Se supplementation for a five-week period induced an Se increase from to 2- to 4-fold in loin and hindleg meat (Dokoupilová *et al.,* 2007; Ebeid *et al.,* 2013; Matics *et al.,* 2017).

Table 5: Effect of dietary supplementation with VE or EcoE on α-tocopherol and selenium content in feed and in minced meat.

		Dietary groups						
Item		CTRL	VE100	VE200	EcoE100	EcoE200	SEM	*P*-value
αT (mg/kg)	Feed	85.00[a]	132.40[b]	253.00[c]	90.00[a]	86.80[a]	5.21	<0.001
	Meat	2.78[a]	4.22[b]	4.60[b]	3.04[a]	3.16[a]	0.27	<0.001
Se (mg/kg)	Feed	0.096[a]	0.094[a]	0.095[a]	0.250[b]	0.395[c]	0.042	<0.001
	Meat	0.174[a]	0.165[a]	0.175[a]	0.191[b]	0.203[b]	0.015	<0.001

αT: α-Tocopherol; Se: Selenium. Values are given as means (n=5). CTRL: control diet (BD: basal diet); VE100: BD supplemented with 100 mg of Vitamin E per kg of feed; VE200: BD supplemented with 200 mg of Vitamin E per kg of feed; EcoE100: BD supplemented with 100 mg of EconomasE™ per kg of feed; EcoE200: BD supplemented with 200 mg of EconomasE™ per kg of feed. SEM: standard error of mean.

[abc]Different letters within the same row indicate significant differences ($P<0.05$) using a one-way ANOVA, Student-Newman-Keuls test.

Meat quality

No statistically significant differences were observed among the five dietary groups regarding the LL proximate composition (Table 6).

The pH and colour parameters of raw LL muscle 24 h *post-mortem* were not substantially different in the five dietary groups (Table 6). Our results on the pHu agree with those of Dalle Zotte *et al.* (2005).

The differences observed in the colour parameters of the cooked LL muscle were not relevant (Table 6).

In LT, LL and BF cooked muscles, the cooking losses and tenderness values were not significantly influenced by dietary treatments. The average values of the cooking losses were equal to 16.73, 19.11 and 13.15% in LT, LL and BF, respectively; while the average values of the WBSF were equal to 16.36, 10.03 and 15.06 N/cm^2 in LT, LL and BF, respectively.

FA composition of LL and BF

The effects of the experimental diets on the FA compositions of LL and BF were reported in Table 7. In general, total lipid content and FA profile were similar in all dietary groups, regardless of the treatment and the tissue considered. The few differences observed only occasionally, small and of little biological value, seem to be attributable to pure randomness rather than to the different diets. The only study on the effect of Se on LL FA composition (Papadomichelakis *et al.,* 2017) showed a very low increase in both 18:2 n-6 and 22:6 n-3 and a more marked increase in Se tissue content, which was not so relevant in our case.

The FA profile also appears similar in the two muscles regardless of the treatment (Table 7). In this regard, only a few studies have investigated the differences in various type of rabbit muscles. Mordenti *et al.* (2010) found some

Table 6: Effect of dietary supplementation with VE or EcoE on proximate composition of *longissimus lumborum* muscle, and on ultimate pH and colour parameters of raw (24 h *post-mortem*) and cooked left muscle.

	Dietary groups						
Item	CTRL	VE100	VE200	EcoE100	EcoE200	SEM	*P* value
Water	73.72	73.87	74.25	74.49	73.96	0.61	0.897
Crude protein	23.38	23.18	23.25	23.06	23.29	0.54	0.995
Total lipid	1.69	1.80	1.34	1.27	1.54	0.15	0.089
Ash	1.21	1.14	1.15	1.17	1.21	0.05	0.703
Energy value (kJ/100 g)	457	461	445	439	453	5.70	0.063
pHu	5.73^{b}	5.62^{ab}	5.54^{a}	5.71^{b}	5.61^{ab}	0.04	0.017
Colour LL raw meat							
L*	49.71	50.22	50.34	51.17	51.51	0.66	0.319
a*	1.03	0.14	0.45	0.61	1.11	0.39	0.392
b*	2.77	2.70	3.04	3.00	3.43	0.26	0.311
Hue	71.85	88.48	82.92	83.14	72.82	6.51	0.313
Chroma	3.20	2.99	3.19	3.20	3.75	0.29	0.467
Colour LL cooked meat							
L*	78.58	78.71	78.56	78.56	78.13	0.41	0.347
a*	–0.25	–0.55	–0.42	–0.75	–0.24	0.34	0.192
b*	10.27^{a}	10.91^{b}	10.21^{a}	11.21^{b}	10.99^{b}	0.21	0.001
Hue	107.37	102.15	109.69	95.85	90.89	11.97	0.417
Chroma	10.33^{a}	11.00^{b}	10.29^{a}	11.27^{b}	10.99^{b}	0.18	0.001

pHu: ultimate pH; LL: *longissimus lumborum*; L*: lightness; a*: redness; b*: yellowness. Values are given as means (n=10). CTRL: Control diet (BD: basal diet); VE100: BD supplemented with 100 mg of Vitamin E per kg of feed; VE200: BD supplemented with 200 mg of Vitamin E per kg of feed; EcoE100: BD supplemented with 100 mg of EconomasE™ per kg of feed; EcoE200: BD supplemented with 200 mg of EconomasE™ per kg of feed. SEM: Standard Error of Mean.

[ab]Different letters within the same row indicate significant differences ($P<0.05$).

Table 7: Effect of dietary supplementation with VE or EcoE on fatty acid (FA) composition and nutritional indexes of *longissimus lumborum* and *biceps femoris* right muscles.

FA content	Dietary groups[2]												
	CTRL		VE100		VE200		EcoE100		EcoE200			*P* value	
(% of total FA)	LL	BF	LL	BF	LL	BF	LL	BF	LL	BF	SEM	tissue	group
C13:0	0.05^{a}	0.30^{c}	0.05^{a}	0.36^{c}	0.05^{a}	0.17^{b}	0.05^{a}	0.04^{a}	0.04^{a}	0.39^{c}	0.03	<0.001	<0.001
C14:0	1.44	1.62	1.71	1.50	1.52	1.54	1.51	1.58	1.57	1.43	0.05	0.617	0.329
C15:0	0.33	0.36	0.36	0.35	0.34	0.34	0.33	0.36	0.35	0.34	0.01	0.155	0.248
C16:0	23.92	23.97	24.93	24.17	24.37	24.32	24.53	25.14	24.54	24.52	0.35	0.761	0.222
C17:0	0.39	0.42	0.42	0.42	0.37	0.46	0.42	0.43	0.42	0.39	0.02	0.051	0.732
C18:0	6.73	6.50	6.86	6.88	7.00	6.74	6.79	6.66	6.59	6.92	0.12	0.895	0.141
ΣSFA	32.96	33.17	34.32	33.69	33.67	33.57	33.50	34.36	33.67	33.98	0.35	0.424	0.076
C14:1 *n*-5	0.12	0.16	0.12	0.15	0.13	0.15	0.10	0.13	0.15	0.12	0.03	0.055	0.312
C16:1 *n*-7	2.71	2.87	2.52	2.34	2.53	2.68	2.46	2.82	2.68	2.48	0.12	0.423	0.058
C17:1 *n*-7	0.21	0.28	0.21	0.24	0.21	0.24	0.25	0.22	0.27	0.22	0.01	0.444	0.383
C18:1 *n*-9	26.42	26.27	26.40	25.52	25.77	26.20	26.18	26.20	26.06	25.52	0.31	0.435	0.269
C18:1 *n*-7	1.38^{b}	1.28^{a}	1.25^{a}	1.23^{a}	1.34^{b}	1.20^{a}	1.40^{b}	1.19^{a}	1.27^{a}	1.21^{a}	0.03	<0.001	0.041
C20:1 *n*-9	0.35	0.34	0.34	0.38	0.32	0.36	0.33	0.33	0.31	0.36	0.02	0.300	0.353
ΣMUFA	31.19	31.21	30.84	29.83	30.30	30.83	30.73	30.88	30.74	29.91	0.35	0.302	0.070
C18:2 *n*-6	25.57	26.50	25.86	26.90	25.98	26.41	25.78	26.27	25.73	26.11	0.35	0.104	0.715
C18:3 *n*-3	1.18^{a}	1.26^{b}	1.26^{b}	1.26^{b}	1.16^{a}	1.31^{b}	1.19^{a}	1.28^{b}	1.17^{a}	1.14^{a}	0.03	0.001	0.012
C20:2 *n*-6	0.54^{a}	0.55^{a}	0.50^{a}	0.59^{b}	0.54^{a}	0.57^{a}	0.55^{a}	0.53^{a}	0.51^{a}	0.61^{b}	0.02	0.001	0.784
C20:3 *n*-6	0.53^{a}	0.47^{a}	0.41^{b}	0.50^{a}	0.49^{a}	0.44^{a}	0.49^{a}	0.43^{a}	0.47^{a}	0.53^{a}	0.04	0.654	0.140
C20:4 n-6	4.74^{a}	3.98^{b}	4.47^{b}	4.40^{b}	5.00^{a}	4.15^{b}	4.94^{a}	4.00^{b}	4.96^{a}	4.73^{a}	0.23	<0.001	0.050
C20:5 n-3	0.15^{a}	0.15^{a}	0.09^{b}	0.11^{ab}	0.15^{a}	0.11^{ab}	0.12^{a}	0.11^{a}	0.13^{a}	0.10^{ab}	0.01	0.258	<0.001
C22:4 n-6	1.78^{a}	1.47^{a}	1.23^{b}	1.56^{a}	1.52^{a}	1.40^{a}	1.62^{a}	1.19^{b}	1.56^{a}	1.64^{a}	0.08	0.045	0.004
C22:4 n-3	0.64	0.51	0.52	0.54	0.57	0.49	0.56	0.46	0.53	0.58	0.03	0.064	0.282
C22:5 n-3	0.55	0.52	0.45	0.49	0.49	0.44	0.51	0.41	0.50	0.50	0.03	0.105	0.085
C22:6 n-3	0.23^{a}	0.23^{a}	0.13^{b}	0.13^{b}	0.14^{b}	0.27^{a}	0.18^{a}	0.21^{a}	0.16^{a}	0.19^{a}	0.03	<0.001	<0.001
ΣPUFA	35.88	35.64	34.85	36.48	36.03	35.60	35.81	34.77	35.85	36.13	1.20	0.885	0.592
ΣPUFA/ΣSFA	1.09	1.08	1.02	1.08	1.07	1.06	1.07	1.01	1.08	1.06	0.02	0.751	0.341
Σn-6	33.16	32.96	32.48	33.95	33.52	32.98	33.38	32.42	32.23	33.62	0.42	0.912	0.776
Σn-3	2.73^{b}	2.67^{a}	2.44^{a}	2.53^{a}	2.51^{a}	2.62^{a}	2.55^{a}	2.45^{a}	2.50^{a}	2.51^{a}	0.07	0.762	0.009
Σn-6/Σn-3	12.19^{b}	12.38^{b}	13.38^{a}	13.45^{a}	13.48^{a}	12.64^{a}	13.18^{a}	13.25^{a}	13.36^{a}	13.50^{a}	0.35	0.740	0.009
A.I.	0.44	0.46	0.48	0.46	0.46	0.46	0.46	0.48	0.46	0.46	0.01	0.825	0.169
T.I.	0.79	0.80	0.86	0.82	0.83	0.82	0.82	0.86	0.82	0.84	0.02	0.998	0.125

A.I.: atherogenicity index; T.I.: thrombogenicity index. Values are given as means (n=10). CTRL: Control diet (BD: basal diet); VE100: BD supplemented with 100 mg of Vitamin E per kg of feed; VE200: BD supplemented with 200 mg of Vitamin E per kg of feed; EcoE100: BD supplemented with 100 mg of EconomasE™ per kg of feed; EcoE200: BD supplemented with 200 mg of EconomasE™ per kg of feed. LL: *longissimus lumborum*; BF: *biceps femoris*; SEM: standard error of mean; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

[abc]Different letters within the same row indicate significant differences ($P<0.05$).

differences in FA composition between loin and thigh muscles as a whole. Other authors studied single muscles and highlighted differences between the glycolytic muscles and the oxidative or mixed types. Alasnier and Gandemer (1998), for example, found differences in FA composition of individual phospholipid classes as related to metabolic type of fibre, as evidenced by the higher amount of n-6 and n-3 long chain PUFA in phosphatidyl-ethanolamine (PE) of oxidative muscles compared to that of glycolytic ones. Other studies on fibre-type composition, activity of energy metabolism enzymes and total lipid contents in various types of rabbit muscles (Gondret *et al.,* 2000; Dalle Zotte *et al.,* 2005; Dalle Zotte *et al.,* 2016) highlighted that LL and BF prevalently show characteristics of glycolytic muscles

Table 8: Effect of dietary supplementation with VE or EcoE on peroxidability index and oxidative status (Thiobarbituric acid-reactive substances) in *longissimus lumborum* and biceps femoris right muscles.

	Dietary groups												
	CTRL		VE100		VE200		EcoE100		EcoE200			*P* value	
Item	LL	BF	LL	BF	LL	BF	LL	BF	LL	BF	SEM	tissue	group
PI	64.65	60.95	59.62	62.41	63.41	60.90	64.10	58.36	63.57	63.77	1.27	0.058	0.205
TBARS (mg MDA/kg)	6.70[a]	7.44[a]	3.97[b]	1.79[d]	1.05[c]	2.51[d]	3.67[b]	4.05[b]	0.64[c]	1.52[d]	0.29	<0.001	<0.001

PI: peroxidability index; TBARS: thiobarbituric acid-reactive substances. Values are given as means (n=10). CTRL: control diet (BD: basal diet); VE100: BD supplemented with 100 mg of Vitamin E per kg of feed; VE200: BD supplemented with 200 mg of Vitamin E per kg of feed; EcoE100: BD supplemented with 100 mg of EconomasE™ per kg of feed; EcoE200: BD supplemented with 200 mg of EconomasE™ per kg of feed. LL: *longissimus lumborum*; BF: *biceps femoris*. SEM: standard error of mean.
[abcd]Different letters within each item indicate significant differences ($P<0.05$) using a two-way ANOVA, Student-Newman-Keuls test.

and that glycolytic metabolism increases with age until 11 wk. Our results on FAs at 12 wk of age seem to confirm the histological and metabolic data from the literature, regardless of diet. Finally, despite the higher content of 18:2 n-6 of which the BD was particularly rich, our data showed that the n-6/n-3 ratios were always not much more than ten and in general lower than those reported in other meat types (Dalle Zotte and Szendrő, 2011). These observations, together with AI and TI values (Table 7) which were within the recommended values (Ulbricht and Southgate, 1991), confirmed the good nutritional value of the rabbit meat.

Oxidative stability

VE and Se effects on iron-induced TBARS in LL and BF muscles are reported in Table 8. The antioxidants improved oxidative stability in both muscles, which showed the same tendency towards oxidation, as shown by the lack of relevant differences in FA composition and consequently in PI values (Table 8). The protective effect was dose-dependent in both the VE and EcoE groups, with TBARS values lower than in the control and decreasing with the increasing amount of antioxidant in the feed. The effect on oxidative stability was not parallel to VE or Se content in the meat (Table 3); however, Se and TBARS contents were not seemingly correlated in rabbit liver (Müller *et al.,* 2002).

The role of VE as an antioxidant is well known and some authors have highlighted a parallel positive effect on total PUFA content (Castellini *et al.,* 2001; Lopez-Bote *et al.,* 1997). Although the VE effect on PUFA content did not emerge in this study, similarly to those of other authors (Lopez-Bote *et al.,* 1997; Corino *et al.,* 2007; Mattioli *et al.,* 2017), its protective effect on oxidative stability was strongly confirmed (Castellini *et al.,* 2001; Lo Fiego *et al.,* 2004; Corino *et al.,* 2007), and in the BF already evident at the lowest concentration.

The anti-oxidative role of Se, probably associated with its involvement in the structure of Se- GPx, is still a matter of debate. In rabbit, studies considering the Se effect are few and the results are often contradictory. Rabbits are poorly responsive to dietary Se deficiency, as liver and kidney contain a sufficient level of non-Se-dependent GPx (Lee *et al.,* 1979). Moreover, in various studies, the Se effect on TBARS is considered not to occur in meat, but in plasma or liver (Erdèlyi *et al.,* 2000; Müller *et al.,* 2002; Zhang *et al.,* 2011). Dokoupilová *et al.* (2007) and Marounek *et al.* (2009) found that Se supplementation did not affect the oxidative meat stability, while other authors demonstrated the protective effect in rabbit (Ebeid *et al.,* 2013) and other species (Xiao *et al.,* 2011; Nambapana *et al.,* 2015; Chauhan *et al.,* 2016). Our results showed a strong protective effect of Se, particularly at the higher dose in both muscles.

CONCLUSIONS

The results provided further information on the effect of the two antioxidants tested on the characteristics of rabbit meat. VE or Se dietary supplementation did not affect the growth performance, carcass traits and meat characteristics, confirming its good nutritional value. Lipid content and FA profiles were similar in the fast-twitch glycolytic muscles, LL and BF, confirming their similarities with regard to fibre types and metabolism. Meat oxidative stability was strongly

improved by both antioxidants, regardless of their content in the meat. Further studies might clarify the impact of VE and Se dietary supplementation at doses which are economically sustainable and therefore would normally be included in the formulation of feed for growing and fattening rabbits, increasing their content in meat with the aim of further improving the nutritional characteristics of the rabbit meat.

Conflict of interest: The authors declare that they have no conflict of interest.

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