# Proximate composition, fatty acid profile, and heme iron and cholesterol content of rabbit meat as affected by sire breed, season, parity order, and gender in an organic production system

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ABSTRACT: The study evaluated the effects of sire breed (SB: Vienna Blue (VB) and Burgundy Fawn (BF)), parity order (P:  $1 = \text{nulliparous}, 2 = \text{primiparous}, \ge 3 = \text{multiparous}$ ), slaughter season (SS: spring, summer), and gender (G: males, females) on the meat quality of rabbits reared under an organic production system. They originated from VB and BF sires mated with females derived from a mix of crossbreds (medium- to large-sized breeds). Rabbits were  $46 \pm 6$  days old, they were housed in groups of five in collective cages, fed a pelleted diet, and slaughtered at a live weight of 2.8 ± 0.13 kg. The hind leg meat samples (from 30 VB and 28 BF crossbred rabbits) were divided into two sub-samples: one was freshly packed in plastic bags, and the other was freeze-dried. Samples were stored at -20°C until analysis. The fresh hind leg samples were analyzed for heme iron and cholesterol contents, and fatty acid (FA) profiles. The freeze-dried hind leg samples were analyzed for proximate composition. Moisture and protein contents were affected by SS. The hind leg meat of rabbits slaughtered in summer showed lower moisture (P <0.01), higher protein (P < 0.01), and lower cholesterol (P < 0.05) contents than that of rabbits slaughtered in spring. Meat of rabbits slaughtered in summer had less C14:0 (P < 0.05) and C16:0 FA (P < 0.01) and a higher proportion of total polyunsaturated FA (PUFA) (P < 0.001) due to n-6 FA (P < 0.01). The proportion of total saturated FA, C18:3 n-3 and C20:3 n-6 (P < 0.05), was influenced by gender. The BF crossbreed showed higher levels of total PUFA (P < 0.05) when reared in summer, primarily due to significant differences in C18:2 n-6 (P < 0.01) and C18:3 n-3 (P < 0.01). This study demonstrates that when rabbits are slaughtered in summer, their meat quality is better because the animals require longer time to reach the fixed slaughter weight; the meat is therefore characterized by a higher degree of maturity, with higher total PUFA and lower cholesterol contents.

**Keywords**: *Oryctolagus cuniculus*; sire breed; rearing system; slaughter season; meat fatty acid profile; meat cholesterol content

# **INTRODUCTION**

Today, global rabbit meat production approaches 1.8 million metric tonnes per year, being predominantly concentrated in Asia (48.8%), Europe (28.4%), and the Americas (18.1%) (http://faostat.fao.org/). In Europe, Italy is the major producer of rabbit meat, and rabbit farming represents the fourth largest zootechnical sector (Dalle Zotte 2014). In Italy, the

demand for organically produced rabbits is rising, and because only pure breeds, first-generation crosses, and local populations are allowed, more extensive research on the selection of coloured breeds with good reproductive performance (Dalle Zotte and Paci 2013), live performance, carcass quality (Dalle Zotte and Paci 2014), and meat quality is required.

Rabbit meat offers excellent nutritive and dietetic properties because it is a lean meat that is rich

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in protein with a high level of polyunsaturated fatty acids (PUFAs) and a low cholesterol content (Hernandez and Dalle Zotte 2010; Dalle Zotte and Szendro 2011). As observed in other species used for meat purposes (De Smet et al. 2004; Wood et al. 2008), endogenous and exogenous factors such as genetics, gender, age, weight, growth rate, rearing system, feeding, and technological factors can modify the nutritive and dietetic properties of rabbit meat (Dalle Zotte 2002; Ramirez et al. 2005; Carrilho et al. 2009; Dalle Zotte et al. 2014a, b).

Therefore, the objective of this study was to investigate the effect of sire breed (SB: Vienna Blue and Burgundy Fawn), parity order (P: 1 = nulliparous; 2 = primiparous, ≥3 = multiparous), slaughter season (SS: spring and summer), and gender (G: male and female) on the chemical composition, fatty acid (FA) profile, and cholesterol content of meat from rabbits reared under organic production system. The effects on live performance, carcass traits, physical quality of meat, and muscle fibre properties are reported in Dalle Zotte and Paci (2014) and Dalle Zotte et al. (2016).

## **MATERIAL AND METHODS**

*Ethical statement*. All animals were reared according to the Council Regulation (EC) No. 834/2007 and the AIAB directives on welfare of animals for experimental and other scientific purposes.

Animals and rearing system. The study was conducted on a rabbit farm certified for organic production, located in the Lombardy region, Italy (Azienda Agricola "Noi e la Natura", www.noielanatura.it) in 2004. Vienna Blue (VB) and Burgundy Fawn (BF) sire breeds were chosen to evaluate the productivity of crossbreds obtained from mating them with females derived from a mix of crossbreds of several medium- to large-sized breeds, excluding the white breeds, e.g. California and New Zealand White (Dalle Zotte and Paci 2014). At 46 ± 6 days of age, fifty-eight weaned rabbits of both sexes derived from rabbit does of different parity order (P: 1 = nulliparous, 2 = primiparous, ≥3 = multiparous) were chosen for this study. Thirty animals were derived from Vienna Blue (VB), and 28 were derived from Burgundy Fawn (BF). The animals were housed in groups of five in collective wire cages with plastic slat floors at a density of  $\leq$  8 rabbits/m<sup>2</sup>, and cages were located in a fattening building equipped with a natural ventilation system and supplied with natural lighting, in line with the Council Regulation (EC) No. 1804/1999 and the AIAB directives (http://www.aiab.it/index.php?option=com\_content&view=article&id=402& Itemid=208). The environmental conditions followed the seasonality throughout the experiment, with temperatures and relative humidity ranging from 10 to 30°C and 60 to 75%, respectively. The rabbits were fed an organic pelleted diet. Feed and drinking water were provided *ad libitum*; the FA composition of the diet is reported in Table 1.

Table 1. Fatty acid profile of the organic diet

Fatty acids	Total FAME (%)
Saturated FA	
C8:0	0.005
C10:0	0.009
C12:0	0.029
C14:0	0.121
C15:0	0.066
C16:0	12.44
C17:0	0.106
C18:0	2.85
C20:0	0.301
C21:0	0.049
C22:0	0.266
C23:0	0.116
C24:0	0.144
Others	0.041
Monounsaturated FA	
C16:1 n-9	0.253
C17:1 n-7	0.041
C18:1 n-9	22.7
C18:1, <i>c</i> + <i>t</i> , n-7	1.181
C20:1	0.368
C22:1 n-9	0.066
Others	0.001
Polyunsaturated FA	
C18:2 n-6	47.1
C18:3 n-6	0.166
C18:3 n-3	5.74
C20:2 n-6	0.042
C20:3 n-6	0.000
C20:4 n-6	0.004
C20:3 n-3	0.014
C22:6 n-3	0.014

FAME = fatty acid methyl esters

*Data collection and sampling.* The rabbits were slaughtered during two slaughter seasons (SS): 22 rabbits in spring and 36 in summer) when they reached an average live weight of 2.8 ± 0.13 kg, which corresponded to an age between 106 and 118 days (minimum slaughter age set by the AIAB organic certification is 14 weeks). The slaughter age associated with the different groups is dealt with in Dalle Zotte and Paci 2014. The rabbits were slaughtered in the farm abattoir by electrical stunning followed by severing of the carotid arteries and jugular veins. The slaughtering and carcass dissection procedures followed the World Rabbit Science Association (WRSA) recommendations described by Blasco and Ouhayoun (1993), as reported by Dalle Zotte and Paci (2014). The right hind leg (HL) was deboned, and the raw meat was ground using a knife mill GRINDOMIX GM 200 (Retsch, Haan, Germany) (7000 g for 10 s). Afterwards, each HL meat sample was divided into two sub-samples: the first was packed (fresh) in plastic bags and held at -20°C until further analysis, and the second was freeze-dried (FD) and stored under the same conditions as the fresh sub-sample.

Chemical analyses. The FD HL meat samples were analyzed according to the AOAC (1984) methods for determination of moisture (method 934.01) and ash (method 942.05), whereas protein content, including glucidic molecules and their catabolites (0.25%), was calculated by difference (Ouhayoun et al. 1990). Heme iron content was measured on fresh HL meat samples using the method described by Hornsey (1956) and was expressed as mg/kg of fresh tissue.

The cholesterol content was determined through the absolute quantitative analysis using high performance liquid chromatography (HPLC) (Casiraghi et al. 1994). A 500 mg meat sample was transferred into a polypropylene tube and then into a 50 ml glass tube with a Teflon cap. To this 50 ml glass tube, 4 ml of 96% ethanol and 2 ml of KOH (50% w/v) was added, and the tube was then placed in a water bath at 70°C for 20 min. The tubes were removed and allowed to cool to room temperature. After cooling, 1 ml of an internal standard (1 mg/ ml solution of pregnenolone) was added to each tube. To each tube, 25 ml of hexane-diethyl ether (ratio of 1:1 v/v) was added, and the tube was then shaken vigorously for 5 min. Subsequently, 20 ml of water was added to the sample, which was stirred for 1 min and then stored overnight at 4°C. The samples were centrifuged at 693.16 g for 20 min. One millilitre of the organic phase supernatant was then removed with a pipette and placed in a Pyrex tube. The sample was then dried in a centrifugal vacuum for 30 min and resuspended in 1 ml of a mobile phase (7% isopropyl alcohol in n-hexane (v/v) solution) for HPLC. The HPLC conditions were as follows: injection volume 20  $\mu$ l; analysis time 9 min; retention time of cholesterol 4 min; internal standard retention time 3.5 min; mobile phase flow 0.8 ml/min; UV detection 208 nm; and chromatography column CLONE BOND  $10 \times 3.9$  mm SILICA 300 micron Phenomenex.

Total lipids were extracted from the homogenized HL meat samples using the Folch et al. (1957) method. Transmethylation was carried out using a blend of methanol, benzene, and sulphuric acid (75:25:4). Gas liquid chromatography was performed on an automated apparatus CE 8000 Top (ThermoQuest Italia S.p.A., Milan, Italy) equipped with a flame ionization detector and an Omegawax® 250 type capillary column (30 m  $\times$  0.25 mm ID) (Sigma-Aldrich, St. Louis, USA). The characteristic operating conditions were as follows: injector temperature 250°C, detector temperature 250°C, hydrogen flow 1.60 ml/min (linear velocity 40.22 cm/s at 200°C). FAs were identified by comparing their retention times to those of authentic FA methyl ester (FAME) standards (Mix C4-24, 18919-1AMP; Supelco, Bellefonte, USA). Results were expressed as a percentage (w/w) of the total FAME.

Statistical analysis. Data were analyzed with an analysis of variance (ANOVA) using the Proc GLM of the SAS software (Version 6, 1990) by including the sire breed (SB: VB, BF), slaughter season (SS: spring, summer), parity order (P: 1, 2,  $\geq$ 3), gender (G), and their interactions. LS means were calculated for all the effects included in the model, and the statistical significance of the differences was assessed with a Tukey's test.

# **RESULTS AND DISCUSSION**

**Proximate composition, heme iron and cholesterol content of hind leg meat.** The effects of sire breed (SB), slaughter season (SS), parity order (P), and gender (G) on proximate composition, heme iron and cholesterol content of HL meat are shown in Table 2. The moisture and protein contents were significantly affected by SS. The HL meat of rabbits slaughtered in summer had lower moisture content

Table 2. Proximate composition (%), heme iron and cholesterol contents (mg/100 g fresh weight) of hind leg meat in rabbits

	Sire breed (SB)		Slaughter season (SS)		Parity (P)		Gender (G)		Significance				DMCE	
	VB	BF	spring	summer	1	2	≥3	female	male	SB	SS	P	G	- RMSE
п	30	28	22	36	31	16	11	23	34					
Moisture	73.7	73.2	73.9	73.1	73.5	73.3	73.7	73.3	73.6	ns	赤赤	ns	ns	0.79
Protein	22.2	22.3	22.0	22.5	22.0	22.3	22.4	22.3	22.2	ns	米米米	ns	ns	0.41
Lipids	2.82	3.26	2.93	3.14	3.21	3.13	2.76	3.19	2.89	ns	ns	ns	ns	0.72
Ash	1.25	1.24	1.24	1.25	1.23	1.26	1.25	1.24	1.25	ns	ns	ns	ns	0.03
Heme iron	0.330	0.320	0.309	0.342	0.325	0.337	0.314	0.314	0.337	ns	ns	ns	ns	0.048
Cholesterol	60.4	63.2	63.6	60.0	61.0	61.9	62.6	63.4	60.2	ns	*	ns	ns	4.9

ns = not significant, RMSE = Root Mean Squared Error, VB = Vienna Blue, BF = Burgundy Fawn \*\*P < 0.01, \*\*\*P < 0.001

(73.1 vs 73.9% for summer and spring, respectively; P < 0.01) and higher protein content (22.5 vs 22.0% for summer and spring, respectively; P < 0.01) than of rabbits slaughtered in spring. The observed difference is likely the result of the higher slaughter age of rabbits reared in summer (118 vs 106 days), as all of the rabbits were slaughtered at the same live weight. In rabbits, when the slaughter age is delayed, the water content of the meat decreases in favour of protein and lipid content (Gondret et al. 1998; Dalle Zotte 2002; Hernandez et al. 2004). Heme iron content did not differ among the experimental factors considered. Compared with hybrid rabbits (Lombardi-Boccia et al. 2002), heme iron was slightly higher in the VB and BF offspring (0.25 vs 0.33 mg/100 g, respectively), likely due to the higher slaughter age as a consequence of the less precocious genotypes.

The SS significantly (P < 0.05) affected the cholesterol content of the HL meat, which was lower in rabbits slaughtered in summer (60.0 vs 63.6 mg/100 g). Again, this difference depends on the slaughter age, as it was observed that cholesterol content decreases with increasing age of the rabbit (Dalle Zotte 2002). Sire breed, parity, and gender did not markedly affect these quality variables.

FA profile of hind leg meat. Table 3 provides the FA profile of the HL meat. With the exception of C10:0, which was significantly higher (P < 0.05) in the VB sire breed, the FA profile was affected neither by SB nor P. The SS was the most significant factor affecting the FA profile of the HL meat. Rabbits slaughtered in summer exhibited meat that contained less C12:0 (P < 0.05), C14:0 (P < 0.05), and C16:0 (P < 0.01) FA, leading to

a numerically lower saturated FA (SFA) profile (37.8 vs 39.0% total FAME; not significant). Consequently, summer HL meat was characterized by a higher proportion of total PUFA (37.3 vs 34.6% total FAME; P < 0.001) due to the contribution of n-6 FA (34.4 vs 31.9% total FAME for summer and spring SS, respectively; P < 0.01). All rabbits received the same diet – thus the differences in FA were not due to the feeding regime but rather to differences in the time to reach the fixed slaughter weight. During the summer SS, the rabbits showed a significantly longer fattening period (+12 days) (Dalle Zotte and Paci 2014), and this delay increased the PUFA content (Parigi Bini et al. 1992; Wood et al. 2008; Lazzaroni et al. 2009), despite a similar intramuscular lipid content between groups. As reported by Hernandez et al. (2008), the differences observed in FA composition were not dependent only on the lipid content of the meat but also on the rabbits' growth rate and age. No data are available in the literature to corroborate the relation between season and FA profile, or between environmental temperature and FA profile in growing rabbits, as all reported data involve the concomitant use of different diets. Nevertheless, heat stress could be a possible indirect factor, as it reduces live performance.

Gender (*G*) also influenced the proportion of total SFA (P < 0.05), the proportion of certain PUFAs, and the ratio of SFA/unsaturated FA (UFA) (P < 0.05). Females produced HL meat with a higher C18:3 n-3 content (P < 0.05) and lower levels of C16:0 (P < 0.05), C20:3 n-6, other PUFAs (P < 0.05), SFA, and the SFA/UFA ratio than males (P < 0.05). De Smet et al. (2004) also reported a gender effect

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Table 3. Fatty acid (FA) profile (% total FAME) of hind leg meat in rabbits

RMSE 0.029 0.049 0.268 0.048 0.009 0.075 0.072 96.0 0.052 0.037 0.014 0.59 0.096 0.172 0.067 1.9 0.12 2.12 0.031 0.051 1.31 2.8 3.1  $SB \times$  $SB \times SS$ ns Significance G ns ns Д ns ns ns ns ns ns SS ns SB ns ns ns ns male 0.110 0.180 0.068 0.265 0.112 3.468 0.697 0.106 39.6 0.063 0.235 0.022 0.385 1.95 21.1 1.43 25.1 28.3 0.067 Gender (G) female 0.110 0.068 0.212 0.198 0.009 0.268 0.270 0.068 0.055 0.094 0.4650.699 0.083 22.3 1.36 26.5 0.075 0.411 0.093 37.2 2.26 29.7 2.22 2.87 36.1 23 0.089 969.0 0.076 0.219 0.190 0.018 0.079 0.250 0.078 0.526 0.113 37.8 0.054 21.8 1.85 30.0 2.06 0.311 36.8 0.457 0.0578 Parity (P) 0.186 0.015 0.104 0.46926.0 0.703 0.106 0.092 38.3 0.085 0.233 21.5 25.6 28.6 1.93 0.261 0.362 3.88 0.065 0.491 0.107 36.3 1.99 1.420.071 16 0.218 0.474 969.0 0.093 0.080 0.191 0.012 0.065 0.288 0.310 0.0880.458 0.111 39.2 2.30 21.8 26.4 28.4 1.95 2.94 34.8 Slaughter season (SS) summer 0.115 0.189 0.016 0.558 0.099 0.075 0.4690.717 0.094 0.058 1.80 0.22 1.44 25.4 29.7 0.0641.96 0.2500.364 3.96 spring 0.015 0.079 0.4640.106 0.080 0.088 0.189 0.283 0.89 39.0 0.23 22.2 26.2 28.3 2.00 0.291 3.09 0.071 0.0542.41 1.35 0.069 0.4680.112 0.099 0.078 0.186 0.017 28.9 0.279 0.335 0.096 0.062 Sire breed (SB) 0.695 38.6 0.24 25.8 1.97 0.511 35.8 0.071 1.41 28 0.108 0.068 0.4650.076 0.192 0.013 0.253 0.320 0.084 090.0 0.473 0.098 0.701 7.86 38.2 2.05 21.8 25.8 0.075 26.3 2.00 3.47 33.3 0.21 29.1 36.1 ∑Monounsaturated FA **EPolyunsaturated FA** C22:6 n-3 (DHA) C20:5 n-3 (EPA) Saturated FA Fatty acids C18:1 n-9 C18:2 n-6 C20:3 n-6 C18:3 n-6 C18:3 n-3 Z20:2 n-6 Z20:4 n-6 Z20:3 n-3 C22:5 n-3 C18:1 n-7 C20:1 n-9 C24:1 n-9 n-6/n-3 C14:0 C15:0 C16:0 C17:0 C18:0 C20:0 C21:0 C14:1 C16:1 C17:1 C10:0 C12:0

FAME = fatty acid methyl esters, ns = not significant, RSME = Root Mean Squared Error, VB = Vienna Blue, BF = Burgundy Fawn P < 0.05, \*P < 0.01, \*\*P < 0.001

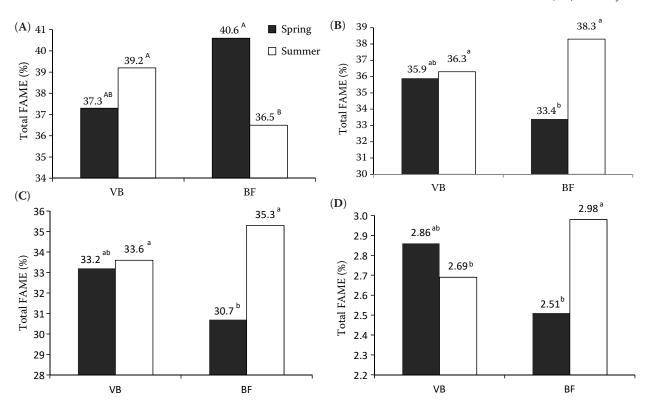


Figure 1. Interaction of sire breed  $\times$  slaughter season on total saturated fatty acids (FA) (**A**), total polyunsaturated FA (**B**), n-6 FA (**C**), and n-3 FA (**D**) of hind leg meat in rabbits

FAME = fatty acid methyl esters, VB = Vienna Blue, BF = Burgundy Fawn  $^{\rm a,b}P < 0.05, ^{\rm A,B}P < 0.01$ 

on the lipid FA profile and suggested a possible effect of sex hormones on the associated enzyme systems.

A significant SB × SS interaction was observed for total SFA (Figure 1A), total PUFA (Figure 1B), n-6 FA (Figure 1C), and n-3 FA (Figure 1D). In the BF crossbreed, the rabbits reared in spring showed a higher SFA content than those reared in summer (P < 0.01), whereas in the VB crossbreed, there was no difference between seasons (Figure 1A). The result observed for the BF group primarily depended on the significantly higher values of C16:0 (P < 0.01) and C21:0 (P < 0.01) FA in the meat of rabbits reared in spring.

As no difference was observed for the proportion of monounsaturated FA (MUFA) among treatments, a significant SB  $\times$  SS interaction emerged for total PUFA, showing higher proportions in the BF crossbreed slaughtered in summer than in spring (38.3 vs 33.4% total FAME, respectively; P < 0.05) (Figure 1B).

The seasonal differences observed in the BF crossbreed for total PUFA depended primarily on

significant differences in the dominant FA: C18:2 n-6 (P < 0.01) and C18:3 n-3 (P < 0.01). This result led to statistically significant differences for total n-6 FA (Figure 1C) and total n-3 FA (Figure 1D).

As reported by other authors (De Smet et al. 2004; Hernandez et al. 2008; Wood et al. 2008; Lazzaroni et al. 2009), age and the degree of fatness may affect FA composition. Indeed, the PUFA content increases with age in rabbits selected for slow growth, and the SFA and MUFA contents increase more rapidly with increasing fatness compared to the PUFA content. Overall, these interactions highlighted a more pronounced susceptibility to changes in the FA profile based on the slaughter season in the BF crossbreed, which might be explained by the lower level of fatness that these animals obtained in summer with a higher age at slaughter (Dalle Zotte and Paci 2014).

A significant SB × P interaction was observed for the proportions of total PUFA (Figure 2A), total n-6 (Figure 2B), and total n-3 FA (Figure 2C). These proportions changed with parity order but changed differently in the two genotypes. In the VB crossbreed,

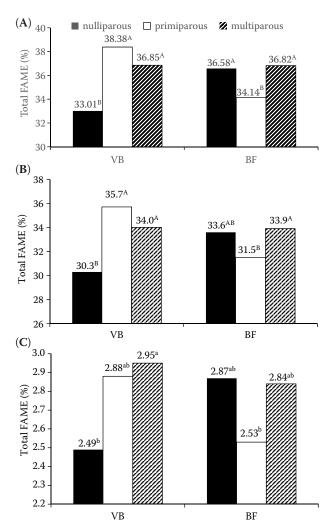


Figure 2. Interaction of sire breed  $\times$  parity order on total polyunsaturated fatty acids (FA) (A), n-6 FA (B), and n-3 FA (C) of hind leg meat in rabbits

FAME = fatty acid methyl esters, VB = Vienna Blue, BF = Burgundy Fawn

 $^{a,b}P < 0.05, ^{A,B}P < 0.01$ 

P2 and  $\geq$ P3 showed significantly higher proportions of total PUFA (P < 0.01), n-6 FA (P < 0.01), and n-3 FA (P < 0.05) than P1 rabbits, while in the BF crossbreed, the P2 rabbits were characterized by a significantly lower proportion of total PUFA than the other parity orders (P < 0.01) as well as a lower total n-6 FA content than the  $\geq$ P3 group (P < 0.01).

These results might be associated with both slaughter age and the degree of fatness. Slaughter age was similar in BF animals of different parities (112 days) and higher in VB P2 (118 days); the latter result explains the high proportion of PUFA observed. With an increase in the degree of fat-

ness, the relative proportion of PUFA decreases; in our study, the VB P1 and BF P2 rabbits were characterized by meat with a higher fat content (lipid 3.2% and 3.7%, respectively; data not shown), and both showed lower values of total PUFA.

### **CONCLUSION**

From the results obtained, it can be concluded that rabbits reared in summer required longer time to reach the fixed slaughter weight than rabbits slaughtered in spring. Consequently, their meat was characterized by a higher degree of maturity with a significantly higher proportion of total PUFA and a lower cholesterol content. The genotypes used did not affect the meat quality, which is consistent with previous results. Gender affected the proportion of total SFA, producing a more favourable SFA/UFA ratio in females. Thus, the crossbred animals derived from the two genotypes showed differences in FA profiles only when subjected to different slaughter seasons and parity order, in which the different degree of fattening and different age at slaughter appear to have played a key role.

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