Meat physical quality and muscle fibre properties of rabbit meat as affected by the sire breed, season, parity order and gender in an organic production system

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MEAT PHYSICAL QUALITY AND MUSCLE FIBRE PROPERTIES OF RABBIT MEAT AS AFFECTED BY THE SIRE BREED, SEASON, PARITY ORDER AND GENDER IN AN ORGANIC PRODUCTION SYSTEM

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Abstract: The aim of the study was to evaluate some meat physical quality and muscle fibre properties of rabbit meat when considering 2 sire breeds (SB: Vienna Blue [VB]; Burgundy Fawn [BF]; both coloured and slow-growing breeds), several parity orders (P: 1, 2, ≥3), gender (G), and 2 slaughter seasons (SS: spring, summer) in an organic production system. The effect of storage time (ST) at frozen state (2 mo at –20°C) of *Longissimus lumborum* (LL) meat was also evaluated. Animals were slaughtered when they reached 2.8 kg of live weight. Then, pH and L*a*b* colour values of *Biceps femoris* (BF) and LL muscles, water loss and Warner-Bratzler shear force of LL and hind leg (HL) meat, and the fibre typing and enzymatic activity of LL muscle were analysed. LL meat from females showed higher b* values than males (0.04 *vs.* –1.25; $P<0.05$). Significant ($P<0.05$) SB×P, SB×G and P×G interactions were observed for the b* value of LL: VB and BF crossbreds presented a higher b* value when born as P≥3 and P2 respectively, VB females showed higher b* value than VB males, and P2 and P≥3 produced males with a significantly lower b* value. HL thawing losses were significantly ($P<0.05$) higher in rabbits slaughtered in summer than in those slaughtered in spring, whereas the opposite result was obtained for LL meat ($P<0.01$). Cooking loss of LL meat was significantly lower in P2 group than P≥3 group ($P<0.05$). The lactate dehydrogenase activity in LL muscle was higher in VB than in BF crossbreds (930 *vs.* 830 IU; $P<0.05$), albeit not supported by differences in fibre type distribution. The ST significantly ($P<0.01$) reduced pH, a* and b* colour values, and increased lightness of LL meat. It was concluded that the crossbreeds derived from VB and BF genotypes and farmed organically did not show remarkable sexual dimorphism, considering their elder slaughter age than rabbits reared under intensive conditions. Physical quality of meat was mainly affected by slaughter season, indicating that in the organic rearing system, specific attention needs to be paid to the farming environmental conditions.

Key Words: rabbit, organic production system, sire breed, slaughter season, meat quality, muscle fibre.

INTRODUCTION

Many factors such as genotype, age, weight, gender, maternal effect, rearing system and temperature can influence growth and change the relative growth of organs and tissues, leading to modifications of carcass traits (Szendrő and Dalle Zotte, 2011), meat quality (Dalle Zotte, 2002) and energy metabolism (Ouhayoun, 1998; Dalle Zotte and Ouhayoun, 1998; Ouhayoun and Dalle Zotte, 1993).

In rabbits the genetic variability among pure-breds is very high, and adult weight and precocity have been demonstrated to have a great influence on growth rate, body composition and meat quality. It has long been demonstrated that the rabbit's weight and age at slaughter markedly influence the meat quality (Ouhayoun and Dalle Zotte, 1993; Ouhayoun, 1998; Dalle Zotte, 2002; Szendrő *et al.,* 2010). Meat quality differences due to gender depend on the slaughter age, as the differences between sexes become more evident as age gradually approaches puberty. In literature, most of

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the data relating to gender effect concerns hybrids selected for fast growth, and they are conflicting as at commercial slaughter age some authors observed significant gender differences on some carcass traits and on meat quality (Pla and Cervera, 1997; Pla *et al.,* 1998), whereas others did not observe any significant difference (Deltoro and López, 1986; Piles *et al.,* 2000; Trocino *et al.,* 2002).

Moreover, it is well known that the birth weight and the rabbit growth are affected by maternal effects, such as litter size, milk production and parity order. The existing literature reported mainly the correlation between birth weight, weaning weight, weight gain and litter size (Dalle Zotte and Paci, 2013), whereas only a few research studied the maternal effect on the meat quality (Szendrő, 2000; Castellini *et al.,* 2003; Metzger *et al.,* 2011).

The effects of alternative rearing systems on the carcass and meat quality of rabbits grown in either intensive or extensive conditions were examined by several authors. Most of them have considered hybrids or genetic lines selected for fast growth (Xiccato *et al.,* 1999; Dal Bosco *et al.,* 2000; Lambertini *et al.,* 2001; Metzger *et al.,* 2003; Cavani *et al.,* 2004; Dalle Zotte *et al.,* 2009; Princz *et al.,* 2009), and less authors used rabbits characterized by slow growth rates (Pla, 2008; D'Agata *et al.,* 2009).

As for the season effect, the literature described mainly the negative effect of high temperatures on live performance and on carcass and meat quality of hybrid rabbits reared under intensive conditions (Chiericato *et al.,* 1993; Marai *et al.,* 2002; Zeferino *et al.,* 2011, 2013), whereas information on crosses of coloured breeds extensively farmed (Dalle Zotte, 2005; D'Agata *et al.,* 2009), or on rabbits reared under organic conditions are still scarce (Pla, 2008; Dalle Zotte and Paci, 2013, 2014; Paci *et al.,* 2014).

In Italy, organic rabbits must be reared according to the guidelines for organic livestock systems (EC Council 1999), and the guidelines of 2 official certification organisms (AIAB, 2001; ICEA, 2007). These guidelines state the use of specific genotypes, housing conditions and feeding system.

The current consumer is looking for safer meats obtained with the respect of the animal welfare, as organic production states, and most of them think that an organic product is characterized by higher nutritional and sensory qualities than conventionally produced food. The organically-obtained rabbit meats have not been deeply investigated for their histochemical, physicochemical, nutritional, and sensory traits (Pla, 2008), and, when considering the Italian guidelines that restrict the use to purebreds (with no red eyes) or their crosses, no information are still available.

This study considered the effect of sire breeds (SB: Vienna Blue and Burgundy Fawn; both coloured and slow-growing breeds), parity order (1=primiparous; 2=secondiparous; ≥3=multiparous), slaughter seasons (SS: Spring and summer) and gender (G: male and female) on meat physical quality and on muscle fibres properties of growing rabbits reared under an organic production system. The effects on doe and litter performance (Dalle Zotte and Paci, 2013), and on growth performance and carcass traits are reported elsewhere (Dalle Zotte and Paci, 2014).

MATERIAL AND METHODS

Animals and housing system

The study was carried out during the spring and summer seasons at a rabbit farm certified for organic production. A detailed description of the farm, feeding, animals and their management is provided in Dalle Zotte and Paci (2014). A total of 58 weaned (at 46±6 d of age) rabbits of both sexes (24 females and 34 males), derived from rabbit does at different parity order (P; 1=primiparous (n=31); 2=secondiparous (n=16); ≥3=multiparous (n=11)), were used. Thirty of them were derived from Vienna Blue (VB) and 28 from Burgundy Fawn (BF) sire breeds (SB). The animals were housed by 5 (≤8 rabbits/m^2) in collective wire cages with plastic slat floor, located in a fattening building supplied with a natural ventilation system and natural lighting, according to the directives of EC Council 1999 and the AIAB (2001). The live performances of the growing rabbits are presented elsewhere (Dalle Zotte and Paci, 2014).

Data collection

Rabbits were slaughtered at 2 slaughter seasons (SS: 22 rabbits in spring and 36 in summer) over the 14th wk of age, at reaching the average live weight of 2.8±0.13 kg. Thus, the average slaughter age corresponded to 106 d for

rabbits slaughtered in spring and 118 d for those slaughtered in summer. The rabbits were slaughtered in the farm abattoir by electrical stunning followed by cutting 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 in the paper of Dalle Zotte and Paci (2014).

Histochemical analysis of the Longissimus lumborum muscle

A total of 24 samples of *Longissimus lumborum* (LL) muscle were used to evaluate morphometric traits of the fibres. Within 10 min after death a small sample of LL was removed from each carcass, frozen in isopentane cooled by liquid nitrogen, and stored at –80°C. For histochemical determinations, 6 serial cross-sections (10 µm thick) from each LL sample were obtained with a cryostat at –20°C. One was stained with azorubine (reference stain). Four sections were processed for the mATPase activity after acid and alkaline pre-incubation (Guth and Samaha, 1970). The sixth was stained for succino-dehydrogenase (SDH) activity according to Nachlas *et al.* (1957). Computerised image analysis (Buche, 1990) was used to classify the fibres as βR (red and slow twitch fibre), αR (red and fast twitch fibre) or αW (white and fast twitch fibre) according to Ashmore and Doerr (1971). The analysis combines the myofibrillar ATPase and SDH stain intensities of each cell.

For each muscle fibre-type the respective percentage, cross-sectional area (CSA, μm^2), compactness index (CI) (perimeter^2/area) and sphericity (SPH) (SD/BD=smallest diameter/largest diameter) were measured.

Enzymatic activity of the Longissimus lumborum muscle

On the same 24 samples of LL muscle used for the histochemical analysis, the equilibrium of the muscular energy metabolism was estimated by measuring the activity of citrate synthase (CS, E.C. 4.1.3.7) and lactate dehydrogenase (LDH, EC 1.1.1.27). These enzymes were respectively characteristics of the oxidative and glycolytic metabolic pathways. To this end, 100 mg of fresh muscle were homogenised in phosphate buffer-pH 7.5 (0.1 M) added with EDTA (2 mM). Enzyme activities were recorded on a Perkin-Elmer Lambda 900 spectrophotometer at 30°C during 5 min after 2 min of equilibration (λ=412 nm for CS enzyme and 340 nm for LDH enzyme) according to Bass *et al.* (1969). Activities were expressed as µmoles of substrate degraded/min per g fresh tissue.

Rheological measurements

After 24 h chilling the reference carcasses (RC), obtained after removing head, thoracic cage organs, liver and kidneys (Blasco and Ouhayoun, 1993), were cut between the 7th and 8th thoracic vertebra and between the 6th and 7th lumbar vertebra to obtain the loin and the hind parts, which were weighed separately.

Rheological measurements were performed on the LL side not used for histochemical and enzymatic analyses, and on the left hind leg (HL) meats: Twenty-four hour *post mortem*, the pH (pH_u) and L*a*b* colour values were measured on LL and on *Biceps femoris* (BF) muscles, whereas Water Holding Capacity (WHC) and Warner-Bratzler Shear Force (WBSF) were evaluated on LL and HL.

The pH_u was measured in duplicate by using a combined Ingold electrode (406 M3) and L*a*b* colour values were assessed, in duplicate, on the surface of raw BF (No.=58) and LL (No.=58) muscles. A Minolta CR100 chromameter (Minolta, Osaka, Japan) was set to the L* (lightness), a* (redness), b* (yellowness) scale (CIE, 1976). Thereafter the meat samples were packed in plastic bags, then sealed and frozen at –20°C for 2 mo. After overnight thawing at room temperature, the meat samples were weighed, pH and L*a*b* colour values were measured again on LL meat, and subsequently used for cooking loss measurements. For this purpose, samples were individually packed under-vacuum in PVC bags and cooked in a water bath at 80°C for 1 h (LL) or for 2.5 h (HL). Cooked HL and loins were used for the WBSF measurements on round cores (diameter 1.25 cm) sheared perpendicularly to muscle fibre direction with a Warner-Bratzler cell fitted on a universal testing machine. WBSF was calculated by averaging 6 measurements per sample.

Statistical analysis

Data were analysed using the GLM procedure of the SAS (2004) program, by including the sire breed (SB: VB, BF), slaughter season (SS: Spring, Summer), parity order (P: 1, 2, ≥3) and gender (G) effects and their interactions. Least Squares Means (LSM) were calculated for all the effects involved in the model and the statistical significance of the differences was assessed with the Tukey test.

RESULTS AND DISCUSSION

pH and colour of Biceps femoris and Longissimus lumborum muscles

The effects of SB, SS, P and G on physical characteristics of BF and LL muscles are shown in Table 1. The pH_u, L* and b* values observed for BF and LL muscles of the 2 SB organically reared were similar to those found for the same muscles in slow-growing rabbits (local grey population) organically reared indoor (Paci *et al.,* 2014). The lack of differences in pH_u and L*a*b* colour values between VB and BF rabbits derives from their comparable live performance (Dalle Zotte and Paci, 2014).

A significant G effect was observed for the b* colour value of LL muscle ($P<0.05$) measured 24h *post mortem*. Females showed higher b* values than males (Table 1), likely due to their numerically higher proportion of βR fibres and lower LDH activity (Table 4; Ouhayoun and Dalle Zotte, 1993). Gender differences for b* value were observed also by Lazzaroni *et al.* (2009) and Trocino *et al.* (2003), with higher values in females. This result may also depend on the older slaughter age of these rabbits, compared to those intensively reared, when the gender differences become more evident for the approaching puberty, which corresponds approximately to the 13th-14th wk of age (Ouhayoun, 1984). As in our study the crossbreds were slaughtered after 14th wk of age, such gender differences are justified.

A significant ($P<0.05$) SB×P interaction was observed for the b* value of LL (Figure 1); the VB crossbreds presented a higher b* value when born as P≥3 (from multiparous does) and a lower when born as P2 (from secondiparous does), whereas the BF crossbreds showed a higher of b* value when born as P2 and lower when born as P1 (from primiparous does). These differences are difficult to explain, because they are not supported by the SB×P interaction for the live performances and carcass traits found by Dalle Zotte and Paci (2013, 2014).

The effect of the SB×G interaction (Figure 2) on meat b* value revealed differences attributable to the gender only in the VB crossbreds, where females showed higher b* value than males ($P<0.05$). In this case, the difference cannot be

Table 1: Effect of sire breed (SB), slaughter season (SS), parity order (PO) and gender (G) on pHu and L*a*b* colour of *Biceps femoris* and *Longissimus lumborum* muscles measured 24 hours *post mortem.*

	SB		SS		P			G		Significance				RMSE
	VB	BF	Spring	Summer	1	2	≥3	Female	Male	SB	SS	P	G	
No. rabbits	30	28	22	36	31	16	11	24	34					
Biceps femoris muscle:														
pH_u	5.85	5.82	5.80	5.86	5.85	5.85	5.80	5.82	5.84	ns	ns	ns	ns	0.12
L^*_{24h}	53.2	53.4	53.1	53.5	53.2	52.7	54.0	53.2	53.4	ns	ns	ns	ns	2.12
a^*_{24h}	3.76	4.10	3.93	3.93	4.37	3.60	3.82	3.75	4.11	ns	ns	ns	ns	1.53
b^*_{24h}	2.00	2.48	2.42	2.06	2.56	2.00	2.15	2.20	2.27	ns	ns	ns	ns	1.32
Longissimus lumborum muscle:														
pH_u	5.69	5.72	5.69	5.72	5.71	5.71	5.70	5.69	5.72	ns	ns	ns	ns	0.08
L^*_{24h}	58.2	58.6	58.2	58.5	57.9	58.4	58.8	58.3	58.5	ns	ns	ns	ns	2.18
a^*_{24h}	2.87	2.62	2.76	2.73	2.78	2.66	2.79	3.07	2.42	ns	ns	ns	ns	1.30
b^*_{24h}	–0.65	–0.55	–0.70	–0.51	–1.14	–0.58	–0.10	0.04	–1.25	ns	ns	ns	*	1.55

RMSE: root mean square error; ns: not significant; *$P<0.05$.

explained completely by the SB×G interaction, as slaughter age was different for each SB and G (105 d for VB males and 113 d for VB females; 113 d for BF males and 115 d for BF females) and consequently they were characterised by a different degree of maturity.

The P×G interaction indicated that in P2 and P≥3 the LL meat from females had a higher b* value than that of males (P<0.05; Figure 3). Again, this result observed in P≥3 group might be explained by differences in slaughter age, as females were slaughtered at an older age (121 *vs.* 115 d). The results in P2 are less explicable, as the rabbits had similar growth rate and were slaughtered at the same age (118 d). Further research with a larger animal size per treatment is advised to corroborate or refute these results.

Water losses and Warner-Blatzer shear force of hind leg and Longissimus lumborum meat

Table 2 presents the effects of SB, SS, P, G, and Figure 4 the P×G interaction on the hind leg and LL meat water losses and Warner-Blatzer shear force (WBSF). Neither water loss nor WBSF were affected by the SB. If referring to the LL meat, the total water loss (on av. 28.7%) and WBSF (1.2 kg/cm^2) were lower than that found in 2 Hungarian genotypes (Large and Hung: 32.8 and 33.7%, and 3.2 kg/cm^2, respectively; Dalle Zotte *et al.,* 2015), obtained using the same methodological procedures.

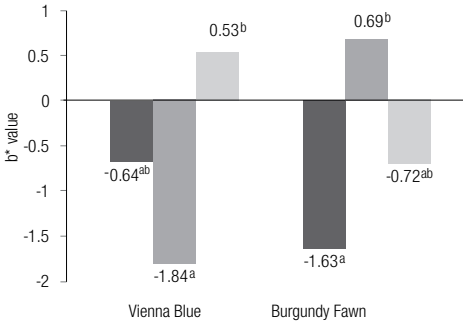

Figure 1: Effect of the sire breed×parity order (P) interaction on b* colour of *Longissimus lumborum* meat. Means with unlike superscripts differ (P<0.05). P1; P2; P≥3.

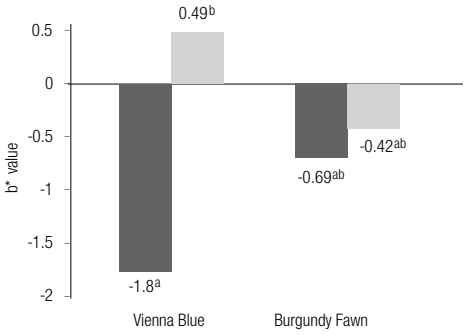

Figure 2: Effect of the sire breed (SB)×gender (G) interaction on b* colour of *Longissimus lumborum* meat. Means with unlike superscripts differ (P<0.01). Male; Female.

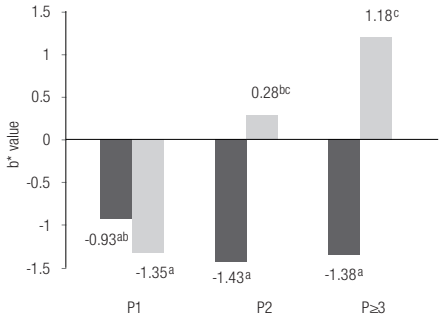

Figure 3: Effect of the parity order (P)×gender interaction on b* colour of *Longissimus lumborum* meat. Means with unlike superscripts differ (P<0.05). Male; Female.

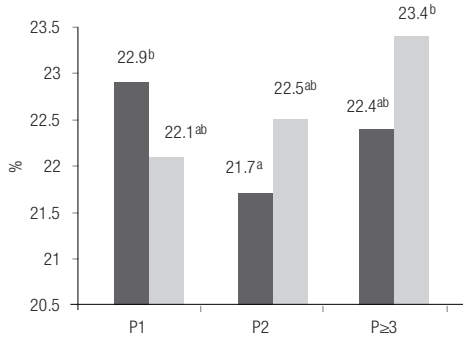

Figure 4: Effect of the parity order (P)×gender (G) interaction on cooking loss of *Longissimus lumborum* meat. Means with unlike superscripts differ (P<0.05). Male; Female.

Table 2: Effect of sire breed (SB), slaughter season (SS), parity order (PO) and gender (G) on water losses (%) and Warner-Bratzler shear force (kg/cm^2) of the hind leg and *Longissimus lumborum* meat.

	SB		SS		P			G		Significance				RMSE
	VB	BF	Spring	Summer	1	2	≥3	Female	Male	SB	SS	P	G	
No. of rabbits	30	28	22	36	31	16	11	24	34					
Hind leg meat measurements (2 mo, –20°C)														
thawing loss	1.33	1.29	1.09	1.53	1.58	1.22	1.12	1.32	1.30	ns	*	ns	ns	0.57
cooking loss	20.9	21.7	21.1	21.4	21.7	20.8	21.2	21.2	21.3	ns	ns	ns	ns	1.74
total loss	22.3	22.8	22.2	22.9	23.4	22.1	22.3	22.5	22.6	ns	ns	ns	ns	1.95
WBSF	1.08	1.11	1.12	1.07	1.06	1.08	1.15	1.12	1.07	ns	ns	ns	ns	0.19
Longissimus lumborum meat measurements (2 mo, –20°C)														
thawing loss	6.24	6.08	7.70	4.62	6.47	6.00	6.01	6.48	5.83	ns	***	ns	ns	1.62
cooking loss	22.4	22.6	22.6	22.4	22.5^{ab}	22.1^{b}	22.9^{a}	22.7	22.3	ns	ns	*	ns	0.95
total loss	28.7	28.6	30.3	27.0	29.0	28.1	28.9	29.1	28.2	ns	***	ns	ns	2.01
WBSF	1.20	1.22	1.08	1.33	1.18	1.15	1.29	1.19	1.23	ns	ns	ns	ns	0.38

WBSF: Warner-Bratzler shear force; RMSE: root mean square error; ns: not significant; *P<0.05; ***P<0.001. Means in the same row and effect with unlike superscripts differ at P<0.05.

The LL meat of rabbits slaughtered in spring exhibited a greater thawing loss than those of rabbits slaughtered in summer (7.70 *vs.* 4.62%; P<0.001). This trend might be explained by the older age (118 *vs.* 106 d) of the summer-slaughtered animals (Dalle Zotte and Paci, 2014), thus confirming the negative rabbit age-meat water loss relationship described in the literature (Gondret *et al.,* 1998; Cavani *et al.,* 2000; Hernández *et al.,* 2004). Surprisingly, an opposite trend was observed for the HL meat (1.09 *vs.* 1.53%, for spring and summer, respectively; P<0.05); however, when considering the total loss, no difference was found due to SS.

The higher water losses obtained for LL meats, compared to the water losses of the HL meat, were mainly due to the partial excision of LL muscle fibres during removal from the carcass. Moreover, the higher amount of cell fibres cut transversally results in an increase in oxidative processes in both lipid and protein fractions during storage, in addition to the tissue lesion caused by the process of freezing and thawing (Dalle Zotte, 2002; Traore *et al.,* 2012). During freezing and thawing, the content and distribution of moisture in meat tissue are altered and ice crystals are formed between and within the fibres. The physical change of water leads to excessive moisture loss during thawing, influencing physical characteristics of meat such as water holding capacity, texture, colour, flavour and appearance (Leygonie *et al.,* 2012a, 2012b).

The P significantly affected cooking loss of LL meat, which was lower in P2 group than P≥3 group (P<0.05). The P×G interaction (Figure 4) showed that P1 and P≥3 produced males and females respectively with a higher cooking loss of LL meat (P<0.05), whereas P2 produced males with lower cooking loss (P<0.05). Overall, the total losses were lower in the considered genotypes (22.6 and 28.7%, for HL and LL, respectively), compared to fast-growing genotypes (27.1 and 34.6% for HL and LL, respectively) (Dalle Zotte *et al.,* 2014), likely due to the higher maturity degree of the organically-farmed genotypes.

Table 3: Effect of storage time (ST) on pH and L*a*b* colour of the *Longissimus lumborum* muscle.

	Storage time (ST)		Significance	RMSE
	24 h at +4°C	2 mo at –20°C	ST	
No. rabbits	58	58		
pH	5.70	5.64	**	0.09
L*	58.2	62.3	***	2.08
a*	2.79	–0.01	***	1.05
b*	–0.35	–2.13	***	1.87

RMSE: root mean square error; **P<0.01; ***P<0.001.

None of the experimental factors considered affected the Warner-Bratzler shear force of the meat, which was similar between the 2 cuts considered (on av. 1.1 and 1.2 kg/cm^2 for HL and LL, respectively), whereas in fast-growing genotypes it tends to be higher in HL than LD meats (0.77 and 0.55 kg/cm^2, respectively; Dalle Zotte *et al.,* 2009). Considering the higher average slaughter age (16 wk) of VB and BF rabbits, compared to the 9 to 12 wk of commercial hybrid rabbits, the meat of the most valued cuts did not increase in toughness, an aspect to consider for the consumer.

Table 4: Effect of sire breed (SB), slaughter season (SS), parity order (PO) and gender (G) on fibre type distribution, morphometric traits and enzymatic activity of *Longissimus lumborum* muscle.

	SB		P		G		Significance			
	VB	BF	1	≥2	Female	Male	SB	P	G	RMSE
No. rabbits	13	11	17	7	9[a]	14				
Fibre type distribution (%)										
αW	81.0	81.0	80.0	82.0	81.1	80.8	ns	ns	ns	4.8
αR	12.4	13.3	12.3	13.4	12.5	13.2	ns	ns	ns	3.6
βR	6.7	5.7	7.6	4.7	6.4	5.9	ns	ns	ns	4.3
Fibre cross-sectional area (μm^2)										
αW	1668	1641	1594	1714	1635	1673	ns	ns	ns	560
αR	893	915	883	924	848	959	ns	ns	ns	349
βR	880	819	836	863	787	912	ns	ns	ns	344
Perimeter										
αW	197	193	192	198	192	199	ns	ns	ns	37
αR	139	141	140	140	135	145	ns	ns	ns	33
βR	139	134	135	139	130	143	ns	ns	ns	30
Compactness index (perimeter^2/area)										
αW	2.27	2.25	2.28	2.24	2.22	2.29	ns	ns	ns	0.18
αR	2.17	2.12	2.21	2.08	2.13	2.16	ns	ns	ns	0.23
βR	2.16	2.14	2.12	2.17	2.10	2.20	ns	ns	ns	0.21
Large diameter (LD) (μm)										
αW	53.5	53.7	53.2	53.9	52.7	54.4	ns	ns	ns	10.2
αR	38.5	38.3	38.5	38.6	36.9	39.9	ns	ns	ns	10.1
βR	39.5	38.4	38.4	39.5	36.8	41.1	ns	ns	ns	8.9
Small diameter (SD) (μm)										
αW	30.0	29.2	28.8	30.4	29.8	29.4	ns	ns	ns	5.7
αR	21.5	21.9	21.1	22.3	21.2	22.2	ns	ns	ns	5.0
βR	21.5	19.9	20.8	20.6	19.8	21.6	ns	ns	ns	4.8
Sphericity (SD/LD)										
αW	0.58	0.57	0.56	0.59	0.58	0.57	ns	ns	ns	0.06
αR	0.58	0.59	0.57	0.60	0.59	0.58	ns	ns	ns	0.07
βR	0.56	0.55	0.57	0.54	0.56	0.55	ns	ns	ns	0.09
Enzymatic activity (IU)[b]										
CS	6.09	6.15	5.94	6.30	6.14	6.10	ns	ns	ns	1.34
LDH	930	830	842	917	865	894	*	ns	ns	125
LDH/CS	160	142	150	151	148	153	ns	ns	ns	38

RMSE: root mean square error.

[a]One sample lost gender identification; ns: not significant; *P<0.05; [b]IU: mmol degraded substrate/min per g of fresh muscle.

*Effect of storage time (ST) on pH and L*a*b* colour of the Longissimus lumborum muscle*

The effect of the storage time (ST) on pH and L*a*b* colour values of LL meat of the slow-growing considered rabbits was evaluated with a double purpose: first, to to reveal the development of pH and colour after 2 mo of frozen storage, and second, to compare these physical values with those from frozen LL meat of hybrid rabbits.

The ST (24 h at +4°C *vs.* 2 mo at –20°C) significantly affected pH and L*a*b* colour values of the LL meat (Table 3). The LL muscles stored for 2 mo at –20°C showed lower pH (P<0.01), higher L* and lower a* and b* colour values (P<0.001) than those stored at +4°C for 24 h.

The pH of frozen meat tends to be lower than unfrozen meat; it depends on the denaturation of buffer proteins and on the consequent release of hydrogen ions, responsible for the pH decrease (Leygonie *et al.,* 2012a). A further

explanation relates to the deamination of proteins by microbial or enzymatic action, with the ensuing release of hydrogen atoms (Leygonie *et al.,* 2011). However, pH has been seen to strongly increase when much longer ST (15 mo) is applied, due to further deamination of proteins, leading to high amount of total volatile nitrogen formation (Dalle Zotte *et al.,* 1998).

The differences for the L*a*b* colour values observed between fresh and frozen meat depend, in part, on the pH values. Given that pH affects myofibril structure, the new organisation is accompanied by colour modifications. The lower the pH, the lighter the meat colour, as the shrinkage of the contractile elements increases the scattering of the light and reduces the importance of myoglobin in selectively absorbing green light (Warriss, 2000; Dal Bosco *et al.,* 2002; Dalle Zotte *et al.,* 2009).

The changes in a* and b* colour values also depend on the alteration of the protein structure, as myoglobin is one of the proteins that denatures during freezing and thawing. The globin fraction of the myoglobin denatures, leading to a loss in colour stability (Añón and Calvelo, 1980; Jeong *et al.,* 2011). The results of a* values confirmed those reported by other authors who found reductions in redness of thawed meat, mainly with increasing storage time, and ascribed these effects to the metmyoglobin produced by the oxidation of myoglobin (Choe *et al.,* 2011).

Fibre characteristics and enzymatic activity of Longissimus lumborum muscle

On average, the fibre type distribution in LL muscle consisted of 81% αW, 12.9% αR and 6.2% βR (Table 4). Compared to the fibre type distribution of hybrid female rabbits (Dalle Zotte *et al.,* 2005), the difference is negligible (78, 17.8 and 4.2%, for αW, αR and βR, respectively) if considering the quite large difference in slaughter age between our slow-growing rabbits and the hybrid ones (112 *vs.* 81 d of age, respectively).

As reported in Table 4, no effect of SB, P or G, and their interactions was found to be significant, excepted for LDH values, which were slightly higher in VB than in BF (930 *vs.* 830 IU; $P<0.05$, respectively). Consequently, these results indicated that sire breed, parity and gender are not able to modify tissue organisation (muscle fibre types, areas or shape, main energetic pathways) in LL muscle. The absence of genetic effect on LL muscle fibres characteristics supports the data reported by Arnal and López (2001) in VB, BF, Champagne d'Argent and Chinchilla breeds. However, another research work (Dalle Zotte and Ouhayoun, 1998) comparing 3 sire strains found the lowest percentage of βR fibres in the breed with the highest degree of maturity. In our study, the 2 crossbreds showed an identical degree of maturity, and this justified the absence of effect on muscle fibre traits. The lack of significance in the main muscle fibre characteristics can therefore explain similar values observed for meat quality traits (pHu, colour, tenderness), which are under the influence of muscle structure and metabolism (Ryu and Kim, 2005; Klont *et al.,* 1998).

CONCLUSIONS

Based on our findings, it could be concluded that the considered genotypes did not substantially differ in their meat quality characteristics, with the exception of the enzymatic activity of LL muscle, which showed higher LDH activity in VB crossbreds independently of the fibre distribution. The crossbreds derived from the 2 sire genotypes showed differences in meat colour only when the effect of sire breed was associated with parity order and gender, likely related to the degree of maturity of the animals as slaughter age changed. Similarly, the differences for the meat water losses observed between seasons seem to be due to the effect of slaughter age. This study provided new information on the meat physical quality of rabbit crossbreds farmed organically, and highlighted the specific possibility of using them even at older ages.

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