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EFFECT OF PASTURE AVAILABILITY AND GENOTYPE ON WELFARE, IMMUNE FUNCTION, PERFORMANCE AND MEAT CHARACTERISTICS OF GROWING RABBITS

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Abstract: To analyse the effect of pasture availability and genotype on innate immunological status, morphological organisation of lymphoid follicles, behaviour, performance and carcass and meat characteristics, 80 growing rabbits (40 Leprino of Viterbo and 40 New Zealand White, NZW) were reared in bicellular standard cages (control) or in a wired pen, provided with an external grass pasture (pasture). Blood samples and behaviour observations (10 rabbits per group) were performed at different ages (weaning, 49 and 89 d). At the end of the trial, 10 rabbits per group were slaughtered to study meat quality/composition and vermiform appendix structure. Leprino rabbits showed higher serum lysozyme values than NZW for all ages and rearing systems. Leprino rabbits showed the highest values in caged animals (29.6 and 32.4 mg/mL, respectively at 49 and 89 d of age), while NZW animals did so in the pen (2.2 and 0.00 g/mL, respectively at 49 and 89 d of age). Haemolytic Complement Assay increased with age in Leprino (43.7 *vs.* 48.6 and 51.2 µg/mL, respectively for cage and pen), but decreased in NZW (68.6 *vs.* 25.8 and 41.1 µg/mL, respectively for cage and pen). Plasma TBARs was always higher in Leprino rabbits (16.0 *vs.* 7.0 µmol malondialdehyde/L at 29 d of age; 17.9 *vs.* 8.0 µmol malondialdehyde/L at 49 d of age; 20.0 *vs.* 11.4 µmol malondialdehyde/L at 89 d of age), whereas plasma tocopherol showed an inverse trend (32.9 *vs.* 46.6 mg/L at 29 d of age; 32.5 *vs.* 44.4 mg/L at 49 d of age; 31.2 *vs.* 42.4 mg/L at 89 d of age). Mortality rate of Leprino rabbits was highest in the cage system, while mortality was highest in NZW housed in the pen. Productive performance, carcass traits and fatty acid composition of meat were strongly affected by genotype and rearing system. Regarding genotype effect, Leprino showed lower daily gains (31.9 and 29.6 *vs.* 44.2 and 40.0 g, respectively for cage and pen system), live (2563 and 2418 *vs.* 2902 and 2650, respectively for cage and pen system) and carcass weights (1561 and 1465 *vs.* 1763 and 1580, respectively for cage and pen system). Pasture availability improved the meat nutritional quality and, in particular, the n-6/n-3 ratio was optimal and the total tocopherol content was suitable to assure a good oxidative stability.

Key Words: rabbit, pasture, innate immunity, behaviour, carcass, meat quality.

INTRODUCTION

Intensive rabbit meat production is mainly based on two- or three-way line crossing of prolific maternal lines and paternal line selected for growth rate and carcass quality (Blasco, 1996; Zgur and Kermauner, 2005). According to recent public opinion surveys, extensive livestock production systems are perceived as offering higher welfare standards and are reputedly more sustainable and environmental friendly (Matthews, 1996). Generally, animals selected for intensive rearing systems do not adapt well to poor and non-standardised environmental conditions (Ferrian *et al.*, 2012). In contrast, local genotypes are more adapted to extensive rearing systems and some pure breeds in danger of extinction could be used assure their preservation.

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Many studies have analysed the effect of alternative rearing systems (Dal Bosco *et al.*, 2000; Metzger *et al.*, 2003; Daszkiewicz *et al.*, 2012) and genetic strain (Paci *et al.*, 2005) on rabbit performance and meat quality, but no studies have examined the immunological and behavioural aspects of animals.

Nowadays there are not enough data and standard rules for extensive and organic rabbit production. Due to this deficit, some national agencies in Italy, Spain, and France laid down their own rules. Italian rules for organic rabbit production impose 35 d as the minimum weaning age for growing rabbits, the use of commercial hybrids is banned and the rearing of minimum 8 rabbits/group and >20 m^2/rabbit of outdoor grassed area are stipulated (Disciplinare per l'allevamento biologico del coniglio, 2001). French guidelines for organic rabbit (Ministere de l'Alimentation, de l'Agriculture et de la Peche, 2010.) set 21 d as the minimum weaning age and the use of commercial hybrids is allowed, with no specification about the minimum number of rabbits per group and 5 m^2/rabbit of outdoor grassed area. The Spanish rules are similar to those of France, the only difference being the recommended weaning age, which is 30 d.

Thus, the aim of this study was to verify the adaptability of 2 rabbit genotypes to pasture availability through the assessment of a wide set of traits (innate immunological status, morphological organisation of lymphoid follicles, behaviour, performance, carcass and meat quality).

MATERIALS AND METHODS

Animals and experimental design

The experimental protocol was planned according to University of Perugia Animal Committee guidelines and the trial was carried out at the experimental farm of the Dept. of Applied Biology (University of Perugia, Italy). Forty weaned 30 d-old Leprino of Viterbo (L) and 40 weaned 30 d-old New Zealand White rabbits (NZW), homogeneous for sex and weight, were assigned to different rearing systems until 90 d of age. Bicellular cages (17 rabbits/m^2) under standard fattening conditions (C); and wired pen (10 rabbits/m^2), with free access to an external grassed paddock of 10 m^2 per rabbit to assure a high grass intake (P).

In total, the following 4 groups (n=20 rabbits) were compared: LC, LP, NZWC and NZWP.

Feeding

Rabbits were fed *ad libitum* an organic diet, certified by national agency. No medical treatment was given. The feed composition was: crude protein 16%, crude fibre 13%, fat 3% and digestible energy 11.0 MJ/kg.

Collection of samples

Grass samples (n=2) were taken from different area of external paddock to evaluate fatty acid composition and tocopherol content. The same analyses were carried out for the feed.

Blood samples (2 mL for each rabbit) were collected from the marginal ear vein of 10 rabbit/group at 29, 49 and 89 d of age. After collection, samples were centrifuged and frozen at –80 °C until analysis.

Live weight and feed intake were recorded every week.

The average feed consumption of the group was used to calculate individual feed:gain ratio.

The mortality was registered and the causes were ascertained by necroscopy.

At 90 d, 10 rabbits/group, with a weight close to the average value of the group (±10%), were selected and slaughtered by cutting the carotid arteries and jugular veins after electrical stunning.

Handling and dissection of chilled carcasses (24 h at 4 °C) were performed as proposed by Blasco and Ouhayoun (1996). The *Longissimus lumborum* (between the 1st and 7th lumbar vertebrae) and *Biceps femoris* muscles were excised from both sides and legs, respectively trimmed of all external fat and epimysial connective tissue and then frozen until analyses.

The gastro-intestinal tract was excised and weighed. Vermiform appendix (VA) was removed from the end of the *caecum* in the same subject and its length, diameter and weight were recorded. The appendix specimens were then placed in a 10%-buffer neutral formaldehyde solution (pH 7.2-7.4) and sent to the Animal Production Department of Madrid University.

Analytical determinations

Lipids of feed, grass and meat (samples of about 5 g) were extracted in a homogeniser with 20 mL of 2:1 chloroform-methanol (Folch *et al.*, 1957), followed by filtration through Whatman No. 1 filter paper. Fatty acids were measured as methyl esters (FAME) with a Mega 2 Carlo Erba Gas Chromatograph, model HRGC (Milano, Italy), using a D-B wax capillary column (25 mm Ø, 30 m long). The fatty acid percentage was calculated with Chrom-Card software. Tocopherol content (α-tocopherol and its isoform β, γ, δ) of feed, grass, serum and meat was quantified by high performance liquid chromatography (HPLC) according to Hewavitharana *et al.* (2004).

The innate immunological status was studied by analysing lysozyme, Serum Bactericidal Activity (SBA) and Haemolytic Complement Assay (HCA). Lysozyme is a strong antibacterial enzyme (against Gram+) that has a synergic action with immune humoral response and serum complement factors; it was measured according to Osserman and Lawlor (1966); the value was expressed in µg/mL. SBA is the main parameter of innate immunity. It is considered a non-specific host defence mechanism and plays an important role in the initial stages of microbial attack; it was determined according to Amadori *et al.* (1997) and its concentration was expressed in %. HCA assesses the risk of infectious disease onset or the severity of already existing pathologies (Barta and Barta, 1993); it was carried out in microtitre plates. Its concentration was expressed in CH50 150/µl. Serum Thio-Barbituric Reactive substances (TBARs) were determined by HPLC using the Halliwell and Chirico (1993) method and expressed as µmol MDA/L.

Muscle TBARs were quantified using the modified method of Tarladgis *et al.* (1960). Ten grams of minced muscle were homogenised for 2 min with 95.7 mL of distilled water and 2.5 mL of 4N HCl. The mixture was distilled until 50 mL were obtained. Five mL of the distillate and 5 mL of TBA reagent (15% trichloroacetic acid, 0.375% thiobarbituric acid) were heated in a boiling water bath for 35 min. After cooling under running tap water for 10 min, the absorbance was measured at 538 nm against a blank. TBARs values were obtained by multiplying optical density by 7.843. Lipid oxidation products were quantified as malondialdehyde equivalents (mg/kg of muscle). Ultimate pH (after 24 h at 4 °C) of *Longissimus lumborum* muscle were measured with a Knick digital pHmeter (Broadly Corp., Santa Ana, CA, USA) after homogenising 1 g of muscle for 30 s in 10 mL of 5 nM iodoacetate (Korkeala *et al.*, 1986).

Vermiform appendix samples were gradually dehydrated with increasing concentrations of ethyl alcohol (50-100%) and embedded in paraffin, sectioned (6 µm) and stained with haematoxylin and eosin. The histological sections were studied. The number, perimeter and area of the lymphoid follicles were determined only in those with complete germinal centre and dome (Castellini *et al.*, 2007).

Ethogram and behaviour observation techniques and calculations

The following behaviours were considered in 10 rabbits per group: stereotypies (biting and smelling bars), eating (feed or grass), motor activities (pasture exploring, jumping, walking and running), static activities (lying down, crouching, sitting-up, staying, standing up on hind legs), comfort (self grooming and scratching), social relationships (smelling, licking, scratching and biting others, attack, dominance, submissiveness features and playing with other rabbits) and escape-alert attempts. The behaviour of rabbits was recorded by 2 operators in the morning (9:00-12:30 am) and afternoon (14:00-17:30 pm) with the focal animal scan sampling method (Martin and Bateson, 1986) during 2 observation cycles before the day of blood sampling (in particular, from 42 till 49 d of age and from 82 till 89 d of age). Before each observation, 5 min were allowed for the animals to adapt to the presence of the operators; during this time, for P groups, the number of indoor animals was recorded. For each behaviour, frequencies from individual rabbits were added together and divided by 10 to give a mean percentage (%) frequency for each observation period. For each rabbit, the percentage of a particular behaviour was calculated as the number of times it occurred divided by the total number of observations and multiplied by 100. Since no differences were found between morning and afternoon and between the 2 cycles of observations, all data were pooled together to obtain a mean value. Each group of rabbits was observed for a total of 1400 minutes (100 min/d×7 d of observations×2 observation cycles).

Statistical Analysis

A linear model considering the single and interactive effects of genotype (n=2), rearing system (n=2) and age (n=3) was used. Least square means were estimated for all immunity traits which were compared by least Significant Differences (LSD) test (STATA, 2005 - procedure GLM).

Non-parametric tests on behavioural patterns were evaluated by X^2 values. For these traits, since the "hour of observation" effect was negligible, data were grouped and the factor omitted from the statistical model.

RESULTS AND DISCUSSION

Fatty acid profile of feed and pasture showed relevant differences in the proportion of main classes of fatty acids (Table 1). Grass pasture has to be considered an important source of energy, protein and vitamins able to reduce the consumption of concentrate in free range rabbits. Grass and standard feed had similar content of polyunsaturated fatty acids, but great differences were observed in linoleic and linolenic acid amounts (Mugnai *et al.*, 2009). In particular, linolenic acid is present in structural lipids in the esterified form, including galactolipids from chloroplasts (Gurr, 1984). Total tocopherol content was higher in pasture than in feed, confirming grass as a relevant source of vitamin E (Mugnai *et al.*, 2009).

Effects of age, genotype and rearing system on rabbit immune and *in vivo* oxidative status are presented in Table 2. Serum lysozyme is a strong antibacterial enzyme (against Gram$^+$) that has a synergic action with immune humoral response and the serum complement (Carroll and Martínez, 1979; Moscati *et al.*, 2008) and is related with macrophage function indicating an inflammation state.

Independently on rearing system, Leprino presented higher Lysozyme than the NZW , which at 89 d of age showed a total absence of this enzyme.

This trend is not easily explicable, however, according to Prieur *et al.* (1974), who described a lysozyme-deficiency in NZW rabbits, it could be argued that the initial lysozyme found in NZW could be due to the maternal milk consumption (Prickett *et al.*, 1933).

HCA is correlated with the risk and/or the severity of infection. HCA is synergic with lysozyme, with cell lysis being facilitated by the presence of lysozyme (Laemmli, 1970).

The HCA showed an opposite trend in the 2 rabbit genotypes: in Leprino the values increased with age, whereas in NZW they decreased. As for lysozyme, being complement activity found in rabbit milk (Rainard, 2003), its initial higher concentration in NZW rabbits could be due to residues from maternal milk.

For both lysozyme and HCA, the decreasing trends found in NZW rabbits could be due to their selection for high performance. Indeed, in livestock animals selection for fast-growing is often accompanied by a reduction in immune responses. In agreement with Ferrian *et al.* (2012), who found a lower immune response in rabbits selected for litter size, a depressive effect of genetic selection on immunity could be hypothesised (Reid, 1995).

The capacity of the serum to inhibit bacterial growth, mainly *Enterobacteriaceae* (Gram$^-$), is expressed by SBA. In this study, SBA showed an unexpected trend and, even in the absence of detectable diseases, seems to indicate an effort

Table 1: Main fatty acids (%) and tocopherols content (mg/kg) of feed and pasture (mean of 3 sampling, at 29, 49 and 89 d of age.

Fatty acid profile	Feed	Pasture
∑ Saturated	19.7	28.4
∑ Monounsaturated	19.5	8.0
C18:2n-6	47.2	13.9
C18:3n-3	11.9	47.7
∑ Polyunsaturated	60.8	63.6
∑ Tocopherols	3.4	26.8

Values are the mean of 3 analyses.

Table 2: Effects of age, genotype (L: Leprino and NZW: New Zealand White) and rearing system (C: bicellular cages and P: wired pen) on rabbit immune and *in vivo* oxidative status.

Age (d)	29		49				89				
Genotype	L	NZW	L		NZW		L		NZW		
Rearing system			C	P	C	P	C	P	C	P	LSD
Lysozyme	25.4^{c}	7.1^{b}	25.3^{c}	22.1^{c}	2.5^{b}	2.2^{b}	32.4^{d}	20.8^{c}	0.00^{a}	0.00^{a}	12.9
SBA	3.7^{a}	2.3^{a}	29.6^{c}	18.9^{b}	26.8^{bc}	35.2^{c}	32.4^{c}	17.8^{b}	20.6^{b}	25.0^{bc}	9.9
HCA	43.7^{ab}	68.6^{c}	25.5^{a}	35.8^{ab}	63.3^{c}	54.0^{b}	48.6^{b}	51.2^{b}	25.8^{a}	41.1^{ab}	5.8
TBARs	16.0^{b}	7.0^{a}	17.3^{b}	18.6^{b}	7.0^{a}	9.1^{a}	19.3^{b}	20.8^{b}	8.1^{a}	14.7^{ab}	3.9
Tocopherols	32.9^{a}	46.6^{b}	33.0^{a}	32.1^{a}	46.8^{b}	42.0^{b}	32.0^{a}	30.5^{a}	45.3^{b}	39.5^{b}	8.9

Lysozyme, µg/mL; Serum bactericidal activity (SBA), %; Haemolytic complement assay (HCA), CH50 150/µl; Thiobarbituric reactive substances (TBARs), µmol malondialdehyde/L and tocopherol, mg/L). No.=10 per group.
$^{a..c}$ Means in the same row not sharing superscript differed significantly (P<0.05).

of adaptation to environmental stress represented by the cage for the Leprino strains and by the free-range system for NZW rabbits.

Serum TBARs was also affected by genotype, being higher in Leprino than in NZW. This was probably due to the higher kinetic activity of Leprino (see behaviour; Table 3) with a consequent increase in oxidative metabolism. Selection for growing traits modifies animal behaviour (Schütz and Jensen, 2001) and NZW rabbits were less active. Consequently, plasma tocopherol showed higher values in NZW animals, probably due to the lower oxidative metabolism which allowed more economical use of antioxidants; in general, the two studied genotypes seemed to have different abilities to store and metabolise vitamin E.

Regarding behaviour (Table 3), NZW rabbits, when reared in the pen, showed the highest percentage of animals located indoors. Stereotypic activities were present only in caged rabbits and among them the NZW showed the lowest behavioural diversity. This finding is in agreement with Dantzer (1986) in swine, who reported a negative correlation between stereotypic activity and behavioural diversity. Gunn Dore and Morton (1995) affirm that the cage rearing system precludes the expression of some basic activities and can lead to atypical behaviours. Barren environments resulted in equipment gnawing (Gunn Dore and Morton, 1995), which may be considered indicative of behavioural abnormalities and a sign of stress (Hansen and Berthelsen, 2000). Pasture access, environmental enrichment or increasing cage size can reduce such stereotypies (Huls *et al.*, 1991, Dal Bosco *et al.*, 2002; Mugnai *et al.,* 2009).

Table 3: Effects of genotype (L: Leprino and NZW: New Zealand White) and rearing system (C: bicellular cages and P: wired pen) on rabbit behaviour.

Genotypes	L		NZW		
Rearing system	C	P	C	P	X^2
% animals indoor	-	5.55^{a}	-	11.92^{b}	2.01
Behaviour					
Stereotypies (%)	5.27^{b}	0.75^{a}	4.17^{b}	0.00^{a}	1.78
Eating (%)	1.69^{a}	11.40^{b}	0.00^{a}	28.62^{c}	2.87
Motor activities (%)	5.28^{b}	17.75^{c}	0.00^{a}	17.52^{c}	2.00
Static activities (%)	44.26^{a}	42.60^{a}	85.79^{b}	36.15^{a}	2.97
Comfort (%)	24.91^{d}	16.48^{c}	5.59^{a}	10.67^{b}	1.45
Social activities (%)	18.59^{b}	6.11^{a}	4.44^{a}	7.05^{a}	1.54
Escape/Alert (%)	0.00^{a}	4.90^{b}	0.00^{a}	0.00^{a}	1.87
Tonic Immobility (s)	139^{b}	90^{a}	54^{a}	116^{b}	53[1]

No.=10 per group.
$^{a..c}$ Means in the same row not sharing superscript differed significantly (P<0.05).
X^2: chi-square.
[1]SEM.

Eating behaviour was also affected by rearing system and genotype: caged animals ate only feed, whereas when pasture was available, rabbits ate mainly grass. NZW rabbits compared to Leprino ate for a longer time and this could be due to their selection for productive performance, which chooses animals with a great appetite and food intake (Grandin and Deesing, 1998).

As expected, the motor activities of rabbits were higher when pasture was available. In general Leprino showed a higher kinetic aptitude, confirming the fact that animals not selected for productive performance show higher kinetic behaviour than those selected for production efficiency (Rauw *et al.*, 1998).

According to Dore and Morton (1995), static activities were the most represented in NZW rabbits reared in cages and were mainly represented by crouching and laying postures (54.25 *vs.* 22.64% and 20.48 *vs.* 3.33%, respectively for crouching and laying in NZW and Leprino rabbits, data not shown).

In Leprino –a kinetic animal– a high rate of comfort behaviours was observed in animals housed in cages, which could indicate a lack of environmental stimuli (Hansen and Berthelsen, 2000); the same authors observed that grooming activity in the cage decreased when the rabbits had access to hay (Berthelsen and Hansen, 1999).

Escape-alert attempts were only observed in Leprino rabbits and was representative of its higher reactivity and more natural behaviour. As rabbits are prey animals, they tend to be on the alert for danger at all times, especially when reared in pasture. This finding was in agreement with the tonic immobility test: Leprino rabbits showed a higher reactivity when reared in the pen, whereas NZW ones reacted faster when reared in cages, confirming that stressful stimuli positively affect TI (Miranda *et al.*, 2006).

NZW rabbits showed the best productive performance (Table 4) both in cage and pen compared to Leprinos. According to other *in vivo* parameters, the discomfort of Leprino in cages was confirmed by its mortality rate: highest for caged Leprino (30 *vs.* 15%, for cage and pen, respectively) and lowest for caged NZW (15 *vs.* 10%, respectively for pen and cage rearing systems; data not shown). Rabbits died from digestive diseases, showing symptoms such as low body weight, bloated abdomen and diarrhoea.

As expected, both genotypes reared in cages showed better performance in terms of slaughter weight, daily gain and feed intake, due to the lower kinetic activity (Lambertini *et al.*, 2001; McNitt *et al.*, 2003; Pinheiro *et al.*, 2008), which reduced the energy consumption while simultaneously increasing the time spent eating. Moreover, Hernández and Gondret (2006) reported that voluntary ingestion in alternative systems does not entirely cover growth requirements due to the time spent on activities other than feeding. Some authors found lower slaughtering weight when studying the effects of exercise on growth (Gondret *et al.*, 2005).

Table 4: Effects of genotype (L: Leprino and NZW: New Zealand White) and rearing system (C: bicellular cages and P: wired pen) on rabbit performance, carcass and meat characteristics.

Genotypes	L		NZW		
Rearing system	C	P	C	P	SEM
Performance					
Slaughter weight (g)	2563^{b}	2418^{a}	2902^{d}	2650^{c}	154.0
Average daily gain (g)	31.9^{b}	29.6^{a}	44.2^{d}	40.0^{c}	5.21
Feed intake[1] (g)	126^{b}	98.7^{a}	132^{b}	142^{c}	21.0
Feed efficiency	3.9^{d}	3.3^{b}	3.0^{a}	3.6^{c}	0.9
Carcass characteristics					
Chilled carcass weight (g)	1561^{b}	1465^{a}	1763^{c}	1580^{b}	121
Full gastro-intestinal tract weight (g)	462.62^{a}	458.94^{a}	521.78^{b}	493.96^{b}	43.34
Full gastro-intestinal tract weight (%LW)	18.05^{a}	18.98^{b}	17.98^{a}	18.64^{b}	3.93
Dressing out (%)	60.9	60.2	60.8	59.6	5.9
Bone shear force (kg/cm^{2})	11.6^{a}	14.0^{b}	10.0^{a}	14.0^{b}	2.1
pH u	5.9^{b}	5.6^{a}	5.8^{ab}	5.7^{ab}	0.5
Meat shear force (kg/cm^{2})	2.9^{ab}	3.4^{b}	2.5^{a}	3.4^{b}	0.4

No.=10 per group. SEM: standard error of the means.

$^{a..d}$ Means in the same row not sharing superscript differed significantly ($P<0.05$).

[1] Only pelleted diet.

Rearing rabbits in bicellular cages gives an advantage to farmers in terms of high productivity and lower risk of aggressive behaviours (Dal Bosco *et al.*, 2002).

When reared in pasture, NZW rabbits showed a great grass intake, which in turn increases the gastrointestinal tract; however, Leprino had the better feed efficiency under free-range conditions and NZW under cage rearing systems. As expected, slow-growing strain (Leprino) was lighter at slaughtering and this process also reduced fatness and meat:bone ratio (Table 4).

Dalle Zotte and Ragno (2005), analysing the performance of organic rabbit, obtained similar values in Blue Vienna and Burgundy Fawn breeds. Shear force of bone and meat were both affected by rearing system, reaching higher values in organic rabbits. Ouhayoun (1998) reported that a decrease in growth rate enhances the relative growth of early maturing tissues (digestive tract, bone) at the expense of late maturing tissues (muscle and fat). As rabbits are usually slaughtered at a fixed commercial live weight, a slow growth enhances early maturing tissues and decreases late maturing tissues. At the same weight, the skin, digestive tract and bones are more developed in slow-growing rabbits whereas the muscles and adipose tissue are lower. Carcass and meat traits (Dalle Zotte and Ouhayoun, 1998) are also modified when using different breeds as terminal sires (Lukefahr *et al.*, 1983), partially due to the different stage of maturity at which they are slaughtered.

Ultimate pH of *Longissimus lumborum* muscle was lower in pasture rabbits, mainly in Leprino. In accordance with previous findings (Cavani *et al.* 2000; Dal Bosco *et al.*; 2002, Pla, 2008), the higher pH found in caged rabbit indicates a lower glycogen store in muscles, with the result that less lactic acid is formed than in pen-raised rabbit, despite the enhancement of the aerobic catabolism of pyruvate. The presumably greater amount of lactate in *Longissimus lumborum* of pen-raised rabbits could be also due to the consequence of a better response of animals to pre-slaughter stress, thus reducing the glycogen consumption (Andersson *et al.*, 1990). Differences in ultimate pH in muscles of pen-raised rabbits could also evidence an adaptation to physical activity and change of oxidative status and energy metabolism in the animal organism.

The shear force of pen-raised rabbit meat showed the greater values and might have resulted from changes in connective tissue due to the higher motor activity (Dal Bosco *et al.*, 2002).

In young rabbits, the maturity of the gastro-intestinal tract depends on the early intake of solid food, which enhances the enzyme activity and caecal function. In the gut, immune responses are initiated in the inductive sites, which include Payer's patch and the VA. These are additional structures that play an important role in the digestive immune system, containing hundreds of dome-follicles (Fortun-Lamothe and Boullier, 2004). After birth, these tissues develop from non-organised immune regions into follicles which act as an interface between food and microbial flora (Weinstein *et al.*, 1994). The appendix secretes bicarbonate, which probably serves as a buffer for the volatile fatty acids produced by caecal fermentation (Cheeke, 1987).

The morphological organisation of lymphoid follicles in the rabbit VA (Table 5) was normal and similar to that described by other authors (Dasso *et al.*, 2000; Marchetti *et al.*, 2001).

No significant effect of rearing system and genotype was shown for such variables. However, when comparing rabbits that had available pasture with those that did not, the number and area of appendix follicles were close to being significantly higher ($P<0.09$, and <0.06, respectively).

Table 5: Effects of genotype (L: Leprino and NZW: New Zealand White) and rearing system (C: bicellular cages and P: wired pen) on rabbit Vermiform Appendix (VA) and follicle characteristics.

Genotypes	L		NZW		
Rearing system	C	P	C	P	SEM
VA					
Weight (% live weight)	0.31	0.26	0.38	0.38	0.09
Diameter (µm)	1.52	1.37	1.72	1.66	0.31
Length (µm)	11.98	11.60	12.48	12.06	1.32
Follicles					
Number	28.40	31.00	37.16	39.00	2.06
Area (μm^2)	1.20	0.77	1.00	0.99	0.43

No.=10 per group. SEM: standard error of the means.

Lipid levels of meat were affected by genotype and rearing systems (Table 6). In general, NZW rabbits showed a higher muscular lipid content due to their lower kinetic activity, which allows them to store more energy in muscles, and probably also due to the genetic selection for increased precocity of meat-type animals (Dal Bosco *et al.*, 2002).

Longissimus lumborum muscle of pen-raised rabbits always showed lower lipid levels, whereas in *Biceps femoris* the differences affected only Leprino.

The fatty acid profile of both muscles was influenced by genotype and rearing system. According to Hernández and Gondret (2006), the fatty acid profile of rabbit meat comprises mainly saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), whereas monounsaturated fatty acids (MUFA) are less represented. In *Longissimus lumborum*, MUFA lowered in free-range animals (Pla, 2008), while PUFA increased; at the same time the PUFA profile changed and n-6/n-3 ratio lowered. *Longissimus lumborum* of Leprino showed higher percentage of SFA.

Biceps femoris showed higher percentage of SFA in pen-raised rabbits, probably for their higher oxidative metabolism (Gondret *et al.*, 2005). As in *Longissimus lumborum* muscle, the pen-raised animals had lower percentage of MUFA and higher PUFA.

Rabbit meat of all the rabbits showed a PUFA/SFA ratio higher than human recommendation (0.45; Pla, 2008), confirming the good fatty acid profile of rabbit meat and emphasising the grass ingestion of pen-housed rabbits, which in turn enhanced n-3 fatty acids (Forrester-Anderson *et al.*, 2006).

Among the PUFA, linoleic (C18:2) and linolenic (C18:3) are essential fatty acids because animals are unable to synthesise them. Linoleic acid is the n-6 family precursor, whereas linolenic acid is the parent of the n-3 family, especially eicosapentaenoic (EPA) and docosahexaenoic (DHA). A minimum intake of EPA and DHA (500 mg/d) is recommended for prevention in human cardiovascular health (ISSFAL, 2004). The amount of linoleic fatty acid is about 10 times greater in rabbit meat than in beef and lamb and around double that of pork meat (Enser *et al.*, 1988). The amount of linolenic acid is also remarkably abundant in rabbit meat (3%, Hernández and Gondret, 2006) in comparison with other meats: 1.37 lamb, 0.70 beef and 0.95 pork (Enser *et al.*, 1988). In our trial, the highest linolenic acid content was obtained in pen-raised rabbit and mainly in *Longissimus lumborum* of Leprino.

Grass intake decreased n-6 and increased n-3 intake, thus reducing n-6:n-3 ratio. This ratio reached values near 4 in pen-raised rabbits of both genotypes, lower than previously reported in other trials (Dal Bosco *et al.*, 2004; Ramírez *et al.*, 2005).

Table 6: Effects of genotype (L: Leprino and NZW: New Zealand White) and rearing system (C: bicellular cages and P: wired pen) on rabbit lipid content (%), fatty acid composition (%), TBARs (mg malondialdehyde/kg) and tocopherol (mg/kg) contents in *Longissimus dorsi* and *Biceps femoris* muscles.

Muscle	*Longissimus lumborum*					*Biceps femoris*				
Genotype	L		NZW			L		NZW		
Rearing system	C	P	C	P	SEM	C	P	C	P	SEM
Lipids	1.40^{b}	1.23^{a}	1.58^{c}	1.43^{b}	0.55	2.32^{ab}	2.15^{a}	2.62^{b}	2.40^{ab}	0.47
∑ Saturated	41.99^{c}	41.08^{b}	38.81^{a}	38.17^{a}	2.63	37.87^{a}	39.78^{b}	39.09^{b}	41.21^{c}	1.35
∑ Monounsaturated	23.59^{b}	18.53^{a}	22.69^{b}	18.14^{a}	1.81	22.78^{b}	15.26^{a}	22.25^{b}	16.35^{a}	0.79
C18:2n-6	24.12^{a}	25.28^{b}	23.87^{a}	25.41^{b}	2.99	27.19	27.47	26.41	27.71	1.05
C18:3n-3	2.68^{a}	4.91^{c}	2.40^{a}	3.19^{b}	1.19	2.90^{a}	3.92^{b}	2.84^{a}	3.54^{ab}	0.72
∑ Polyunsaturated	34.42^{a}	40.39^{b}	38.50^{b}	43.69^{c}	3.48	39.35^{a}	44.96^{b}	38.66^{a}	42.44^{b}	1.93
∑ n-6 (C≥20)	5.40^{a}	7.31^{b}	8.87^{b}	8.71^{b}	1.85	6.52^{a}	10.08^{b}	6.78^{a}	7.51^{a}	1.74
∑ n-3 (C≥20)	1.50^{a}	2.38^{b}	2.22^{b}	3.20^{c}	1.06	1.66^{a}	3.08^{b}	1.77^{a}	2.98^{b}	1.14
n-6/n-3	6.90^{b}	4.53^{a}	6.67^{b}	4.72^{a}	1.28	6.51^{a}	5.40^{a}	6.74^{a}	5.51^{a}	1.34
TBARs	0.11^{a}	0.13^{a}	0.16^{b}	0.14^{ab}	0.04	0.13^{a}	0.18^{b}	0.22^{c}	0.20^{bc}	0.11
∑ Tocopherols	3.22^{a}	4.15^{a}	3.48^{a}	7.31^{b}	1.50	5.44^{a}	6.31^{ab}	5.48^{a}	8.46^{b}	1.09

No.=10 (per group). SEM: standard error of the means.

$^{a..b}$ Means in the same row not sharing superscript differed significantly ($P<0.05$), for each muscle.

Lipid oxidation is the major non-microbial factor responsible for the deterioration of meat quality. It leads to discoloration, higher drip-loss, and the development of off-odours and off-flavours (Monahan, 2000). Tocopherol is one of the most active muscle antioxidants, so is generally correlated with the extent of lipid oxidation.

Muscle tocopherol content generally increased in pen-raised animals, but the difference was significant only for NZW rabbits, although TBARs values were quite similar in all the groups. Leprino, despite showing a lower tocopherol level, also had a lower TBARs. The discrepancy between the theoretical assumption of a positive correlation between tocopherol and oxidative stability of meat could be explained by the differences in the fat level of the genotypes, which in turn modulates the TBARs value.

Biceps femoris, compared to *Longissimus lumborum*, showed a higher TBARS and tocopherol content according to the higher oxidative metabolism and fat level.

CONCLUSION

Rabbit genotype and rearing system showed major interactions and affected almost all the analysed traits. The slow-growing strain seems more adapted to extensive rearing systems, but in both genotypes pasture improves animal welfare and reduces productive performance. Genotype affected immunological traits and rabbits selected for fast-growing were characterised by a reduction in innate immune responses. Pen-raised rabbits also had a different meat quality (lower pH, lower intramuscular lipids, and higher TBARs), which could be explained by the adaptations to exercise, physiologically relevant to meet the energy demand and reduce muscle reliance on exogenous substrates during exercise. Such changes are more pronounced when slow-growing rabbits are used.

In conclusion, although the pen-rearing system reduced productive performance, it increased meat nutritional quality, lowering lipid content, increasing PUFA and vitamin E and decreasing n-6/n-3.

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