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Effect of genotype and rearing system on the native immunity and oxidative status of growing rabbits

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ABSTRACT - To investigate the effects of genotype and rearing system on innate immunity and oxidative status, 40 weaned commercial hybrid (H) rabbits and 40 rabbits of a local population (LP) were assigned to two rearing systems: cage (17 rabbits/m^2) and pen (1x2 m, 10 rabbits/m^2). Rearing H rabbits in pen resulted in a higher lysozyme concentration ($P<0.05$), an increased bactericidal activity ($P<0.05$) and a lower complement haemolytic activity ($P<0.05$) with respect to those reared in cage. Opposite results were obtained for LP rabbits. In addition, genotype H was more susceptibility to inflammation than LP in pen, whereas genotype LP suffered more in cage. A higher lipid ($P<0.05$) and protein ($P<0.05$) oxidation was found in *Longissimus dorsi* and *Biceps femoris* of rabbits H in pen compared to those in cage. An opposite trend was observed for LP rabbits. In cage, LP rabbits were more susceptible to oxidative stress than H rabbits. Taken together, our results show that immune and oxidative resistance ascribed to a specific genotype are important factors to be considered when a specific rearing system should be adopted. Considering this, rabbit LP seem to better adapt to larger spaces during growing.

Key words: Rabbit, Native immunity, Oxidative stress, Rearing system.

Introduction - The increased interest in less intensive rearing methods has led to implementation of systems aware of animal welfare and without impairing of environmental sustainability, economy and food security. Intensive rearing systems commonly use commercial hybrid selected for their rapid growth rate and adaptability to narrow spaces. However, these genotypes are characterized by a low capacity to cope with certain environmental conditions due to their low immune-competence and high susceptibility to environmental stresses. On the contrary, alternative rearing systems require animal characterized by a slow growth rate, adaptable to various environments, which may lead to favourable productive performance. In recent years, studies carried out on local rabbit populations with a slow growth rate have showed that these animals can achieve a good productive performance, are more rustic and able to cope with different rearing systems (Paci *et al*., 2004). The aim of the present study was to investigate whether the native immunity and oxidative status of two rabbit genotypes are affected by rearing systems.

Material and methods – The trial was carried out at the farm of the Department of Applied Biology, University of Perugia, under controlled environmental temperature (14-20°C) and relative humidity (60-75%). Eighty weaned rabbits (30 days of age) of two genotypes (40 commercial hybrid rabbits H, and 40 rabbits of a local population LP; Paci *et al*., 2004) were homogeneously assigned into two hous-

ing systems: conventional bi-cellular cages (17rabbits/m^2) and wire netted pens (1x2m, 10rabbits/m^2). At 85 day, 10 rabbits per group were sacrificed by cutting the carotid arteries and jugular veins after electrical stunning. Before slaughtering, blood samples were obtained by cardiac puncture for evaluation of the basal immune parameters (lysozyme, bactericidal activity, haemolytic complement) and oxidative status (antioxidant power, AOP, reactive oxygen species, ROS, thiobarbituric acid reactive substances, TBA-RS, and protein carbonyl groups, PCGs). Serum lysozyme was measured by using a lyso-plate assay (Osserman and Lawlor, 1966). Percentage of serum bactericidal activity (BA) was performed according to Amadori *et al*. (1997) method. The haemolytic complement assay was carried out in microtitre plates and values were expressed as CH_{50} (Barta and Barta, 1993). The levels of ROS and AOP were determined by using commercial kits (Diacron, Grosseto, Italy). Plasma TBA-RS were determined by HPLC (Jasco PU 1580) with fluorescence detector (Jasco FP 1520) according to the method of Iorio *et al*. (2007). Plasma PCGs, expressed as nmol/mg protein, were quantified by spectrophotometer (Jasco UV 2075 Plus) after reaction with 2,4-dinitrophenylhydrazine as proposed by Levine (1994). The protein concentration was measured by means of the Bio-Rad protein assay reagent (Bio-Rad, Hercules, CA, USA) according to the Bradford method (1976). After the slaughtering, *Longissimus dorsi* and *Biceps femoris* muscles were excised from the left side of refrigerated carcasses and adequately homogenised with phosphate buffer for the evaluation of tissue TBA-RS and PCGs. TBA-RS were prepared using the method of Ke *et al*. (1977). Chromatographic conditions were the same of those used for the determination of the lipid oxidation products in plasma samples. PCGs were determined spectrophotometrically using the method proposed by Reznick and Parker (1994). Statistical analysis was performed by STATA (2005), using a linear model and taking into account the fixed effects of genotype and rearing system.

Table 1. In vivo immune and oxidative status of rabbits.

	H		LP		SEM
Variable	Cage	Pen	Cage	Pen	
Lysozyme (µg/ml)	12.53a	15.23b	20.64c	12.55a	1.84
BA (%)	11.75a	14.59b	14.75b	10.59a	0.98
Complement CH50	77.83c	64.50b	60.71a	65.83b	3.80
AOP (µmol HClO/ml)	286.52a	308.33b	300.77ab	312.65b	21.54
ROS (nmol H_2O_2/ml)	19.23ab	21.51b	20.06ab	18.11a	1.98
TBA-RS (nmol/ml)	32.75b	33.44b	38.35c	27.90a	1.64
PCGs (nmol/mg protein)	2.37a	2.43ab	3.31b	3.93bc	0.92

a, c: P<0.05.

Results and conclusions – *In vivo* native immunity and oxidative status of H and LP rabbits reared in cage or pen are shown in Table 1.

Native immunity and oxidative status were affected either by the rearing system and/or genotype. In detail, rabbits H reared in pen were characterized by a higher serum lysozyme concentration, an increased bactericidal activity and a concomitant lower complement haemolytic activity when compared to rabbits reared in cage. On the contrary, LP rabbits reared in cage were more susceptibility to inflammation than those reared in pen. Nevertheless, the genotype LP (more rustic) was more resistant to inflammation with respect to genotype H when the density was low. This was also confirmed by the lower plasma levels of TBA-RS and ROS of LP rabbits reared in pen with respect to those obtained by H rabbits reared in the same system. Increased plasma ROS and TBA-RS levels have been frequently associated with the inflammation process (Koner *et al*., 1997). A better adaptability, in terms of immune response, of rustic genotypes to less populated environments has been also demonstrated in our previous study comparing Leprino of Viterbo (more rustic) with White New Zealand rabbits (Mugnai *et al*., 2008).The oxidative status of *Longissimus dorsi* and *Biceps femoris* muscles is reported in Table 2. Lipid and protein oxidation were higher in both muscles of H rabbits reared in pen compared to those reared in cage. This could be due to a higher locomotory activity in pen, inducing oxidative stress, as have been observed

in our previous study (Dal Bosco *et al*., 2002). However, an opposite trend was observed for LP rabbits being more susceptible to oxidative stress in cage than in pen. Moreover, genotype LP reared in cage was more susceptible to oxidative stress than genotype H reared under the same conditions with detrimental consequences on the lipid and protein fraction of both muscles. LP rabbits, being more rustic, are used to move more and may suffer undergoing injuries when the available space is limited. Compared to genotype H, the higher oxidation of muscle lipids and proteins of genotype LP reared in cage might have contributed to the higher plasma levels of the respective parameters or *vice versa*. A straight correlation between oxidative stress parameters in plasma and tissue has been also found by others (Powers and Leeuwenburgh, 1999). These results show that immune response and oxidative status are closely correlated to the genotype and rearing system. Rustic genotypes seem to be more adaptable to low density and with wider space rearing systems because of their higher resistance to inflammation and oxidative stress.

Table 2. Oxidative status of *Longissimus dorsi* and *Biceps femoris* muscles of rabbits.

	H		LP		SEM
	Cage	Pen	Cage	Pen	
Longissimus dorsi					
TBA-RS (mg/Kg)	0.22a	0.32b	0.33b	0.29b	0.06
PCGs (nmol/mg protein)	3.34a	5.05b	5.33b	4.52b	1.05
Biceps femoris					
TBA-RS (mg/Kg)	0.35a	0.50b	0.41ab	0.42ab	0.11
PCGs (nmol/mg protein)	6.64a	11.94c	11.50c	8.07b	0.97

a, c: P<0.05.

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