# PASTURE AVAILABILITY AND GENOTYPE EFFECTS ON RABBITS: 1. HEALTH AND WELFARE

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## ABSTRACT

To analyze the effect of pasture availability and genotype on health and welfare 80 growing rabbits (40 Leprino of Viterbo and 40 New Zealand White, NZW) were assigned to two groups: control, reared in bicellular standard cages or wired pen, provided of an external grass pasture. Blood samples and behaviour observations (10 rabbits per group) were performed at different ages (weaning, 49 and 89 days). Lysozyme presented at all ages and both housing systems a significant genotype effect: Leprino rabbits showed higher values than NZW rabbits. Serum Bactericidal Activity (SBA), on the contrary, was influenced by housing systems in both genotypes: Leprino showed the highest values in caged animals, while NZW in pasture ones. Haemolytic Complement Assay increased with age in Leprino, while it decreased in NZW. Plasma TBARs (Thio-Barbituric Acid Reactive substances) were always higher in Leprino rabbits whereas plasma tocopherol showed an inverse trend. Stereotyped activities were present only in caged rabbits and NZW showed the highest incidence at a young age. Eating was affected by housing system and genotype, NZW pasture rabbits showing a preference for grass. As expected, animals with pasture availability showed the highest percentage of motor activities. Regarding comfort behaviours, at a young age its percentage was higher in caged animals, while at older ages it was highest in Leprino rabbits. NZW caged rabbits showed the highest percentage of static behaviours in old age. Leprino rabbits showed more social behaviours than NZW. Mortality rate of Leprino rabbits was the higher in cage housing, while in NZW this parameter was highest in the pasture system.

**Key words**: Rabbit genotype, Pasture, Health, Welfare.

## INTRODUCTION

A correct assessment of animal welfare should involve multiple indicators such as behaviour, physiology, body injuries, disease and performance (Broom, 1997). In recent years it has become evident that there is a strong link between animal behaviour, stress and the neuro-endocrine and immune systems (Straub *et al*., 2000; Marchetti *et al*., 2001). Therefore, unfavourable environmental conditions could lower homeostatic functions, such as the immune response and in particular the innate immune system (Amadori *et al*., 1997). This aspect of the immune system is affected by environmental stressors and has a significant correlation with the health status of animals (Moscati *et al*., 2003).

Moreover, most of the intensive housing and management systems used in commercial rabbit farms are not the best with respect to the ethological needs of animals. Single caging isolates rabbits and prevents them from physical and visual contact and social interaction, particularly if solid-walled cages are used (Gunn-Dore, 1994). Furthermore, spatial restriction precludes the expression of some basic activities (Gunn-Dore and Morton, 1993) which can lead to atypical behaviours, indicative of frustration, anxiety or boredom (Gunn-Dore, 1994), and to skeletal anomalies (Lehman, 1991). For these reasons much attention needs to be given to alternative housing systems that promote

appropriate, environmentally-friendly and healthy rearing of rabbits in terms of both physiology and behaviour.

The aim of the present study was to define a range of standard values for some traits of innate immunity, plasmatic TBARs (Thio-Barbituric Acid Reactive substances), Tocopherol and some behavioural traits in rabbits from different genotypes subjected to different housing conditions.

## MATERIALS AND METHODS

### Animals and experimental design

The trial was carried out on the experimental farm of the Dept. of Applied Biology (University of Perugia, Italy). Eighty weaned (30 d) Leprino of Viterbo (L) and New Zealand White rabbits (NZW), were divided in two homogeneous groups (sex, weight and genotype) and assigned to different housing systems:
- bicellular cages (17 rabbits/$m^2$) under standard fattening conditions (C);
- wire pen (10 rabbits/$m^2$), with free access to an external grassed paddock (1 rabbit/20 $m^2$), after a week of gradual adaptation (P).
The mortality was registered and the causes were ascertained.

### Feeding

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

### Blood sampling and analytical determinations

Blood samples (2 ml for each rabbit) were collected (on 20 rabbits per group) from the marginal ear vein at 29, 49 and 89 days of age. After collection, samples were immediately sent to the laboratory where they were centrifuged and frozen at -80 °C until analysis. Serum lysozyme was measured according to Osserman and Lawlor (1966); the value was expressed in µg/mL. The Serum Bactericidal Activity (SBA) was determined according to a previous method validated for cattle (Amadori *et al*., 1997) and its concentration was expressed in %. The Haemolytic Complement Assay (HCA, Barta and Barta, 1993) was carried out in microtitre plates. Its concentration was expressed in CH50 150 $\mu l^{-1}$. TBARS were determined by HPLC according to the method of Halliwell and Chirico (1993) and expressed as µmol MDA/L. The α-tocopherol level of plasma was assessed according to Schuep and Rettenmeier (1994).

### Ethogram and behaviour observation techniques and calculations

10 rabbits/group were marked with different colours on their back and the following behaviours considered: 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 intents. For two observation cycles of one week each (from 42 till 49 days old and from 82 till 89 days old) the behaviours were recorded by 2 operators in the morning (9:00-12:30 am) and in the afternoon (14:00-17:30 pm) with the focal animal scan sampling method (Martin and Bateson, 1986). Before each observation 5 minutes were allowed for the animals to adapt to the presence of the operator, 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, all data were pooled together to obtain a mean value. Each group of rabbits was observed for a total of 1,400 minutes (100 min/day x 7 days of observations x 2 observation cycles).

**Statistical Analysis**

A linear model (STATA, 2005 - procedure GLM) was used; all immune traits were expressed as Least Square means and variability was expressed as LSD, considering the effects of genotype (n=2), rearing system (n=2) and age (n=3). Non-parametric tests on behavioural patterns were done with proc CATMOD and significance was evaluated by $X^2$ values. Since the factor "hour of observation" showed a negligible effect, data were grouped and the factor omitted from the statistical model.

## RESULTS AND DISCUSSION

Innate immunity traits (Lysozyme, SBA and HCA), TBARs and Tocopherol values are shown in Table 1.

**Table 1**: Effects of genotype and rearing system on rabbit immune and oxidative status

| Age (days) | 29 | | 49 | | | | 89 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotype | L | NZW | L | | NZW | | L | | NZW | | |
| Housing 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 |

N=10 per group; a..c: P<0.05

Lysozyme is a strong antibacterial enzyme (against Gram$^{+}$) that has a synergic action with immune humoral response and factors of the serum complement (Carroll and Martinez, 1979). Lysozyme titration is essentially related with macrophage system function and indicates the presence of inflammation. In this trial Lysozyme titration values showed a significant genotype effect and Leprino presented always higher values than NZW. SBA is a major parameter of innate immunity. The capacity of the serum to inhibit bacterial growth is assessed by the presence of complement factors and modulates the concentrations of natural antibodies against more ubiquitous environmental bacterial agents, mainly Enterobacteriacee (Gram$^{-}$). It gives some indications about the defence mechanisms of the animal that activate the complement system. In our study, SBA showed a trend not easily explainable and, even in the absence of visible pathologies, it seemed to indicate an effort to continuously adapt to environmental stress as represented by cage for the less selected rabbit (Leprino) and by pasture for NZW. The HCA is a test that shows complement activity and it helps in assessing the risk of infectious disease onset or the severity of already existing pathologies. The HCA showed an opposite trend in the two analysed genotypes: thus, while in Leprino HCA values increased, in NZW they decreased with age. Low values of HCA indicate a high risk of infection or the severity of an infective pathology already in action. TBARs values also showed a genotype effect, being always higher in Leprino. Plasma tocopherols showed larger values in NZW rabbits. These two parameters showed a negative correlation, i.e.: high TBARs were associated with low plasma levels of tocopherols. The high values of tocopherols in NZW rabbits were probably due to the large pasture intake and the lower motor activity compared with Leprino (28.62 vs. 11.40%, eating grass at 89 days, respectively for NZW and Leprino, data not shown). In general, the two studied genotypes seemed to have different abilities to store and metabolise vitamin E. Behavioural results are shown in Table 2.

**Table 2**: Effects of genotype and rearing system on rabbit behaviour

| Age | | 49 | | | | | 89 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | | L | | NZW | | | L | | NZW | | |
| Housing system | | C | P | C | P | $\chi^2$ | C | P | C | P | $\chi^2$ |
| % animals indoor | | - | 15.00[a] | - | 26.19[b] | 3.70 | - | 5.55[a] | - | 11.92[b] | 2.01 |
| Stereotypies | (%) | 8.58[b] | 0.00[a] | 17.97[c] | 0.00[a] | 1.50 | 5.27[b] | 0.75[a] | 4.17[b] | 0.00[a] | 1.78 |
| Eating | (%) | 3.44[a] | 23.59[b] | 3.05[a] | 41.90[c] | 3.21 | 1.69[a] | 11.40[b] | 0.00[a] | 28.62[c] | 2.87 |
| Motor activities | (%) | 11.94[b] | 29.30[c] | 6.45[a] | 32.97[c] | 2.21 | 5.28[b] | 17.75[c] | 0.00[a] | 17.52[c] | 2.00 |
| Static activities | (%) | 57.89[b] | 24.26[a] | 58.30[b] | 21.39[a] | 3.22 | 44.26[a] | 42.60[a] | 85.79[b] | 36.15[a] | 2.97 |
| Comfort | (%) | 15.07[c] | 9.34[b] | 14.23[c] | 3.03[a] | 1.34 | 24.91[d] | 16.48[c] | 5.59[a] | 10.67[b] | 1.45 |
| Social activities | (%) | 3.07[a] | 11.86[b] | 5.65[a] | 6.25[a] | 0.89 | 18.59[b] | 6.11[a] | 4.44[a] | 7.05[a] | 1.54 |
| Escape/Alert | (%) | 0.00[a] | 1.63[b] | 0.00[a] | 0.71[b] | 0.24 | 0.00[a] | 4.90[b] | 0.00[a] | 0.00[a] | 1.87 |
| Tonic Immobility | (sec) | 86 | 80 | 74 | 52 | 21* | 139[b] | 90[a] | 54[a] | 116[b] | 53* |

N=10 per group; a..d.: P<0.05 (for each age)

At both ages, NZW showed the largest percentage of animals located indoors. Stereotypic activities were present only in caged rabbits and NZW performed the highest percentage of them at both ages. This finding confirms that cage rearing precludes the expression of some basic activities (Gunn-Dore and Morton, 1993), a deficit that can lead to atypical behaviours indicative of frustration, anxiety or boredom (Gunn-Dore, 1994). Eating was also affected by housing system and genotype: caged animals were able to eat only feed whereas when pasture was available rabbits were observed to eat mainly grass. A larger percentage of NZW than Leprino rabbits consumed feeding pasture. As expected, the motor activities of rabbits were higher when pasture was available. Caged Leprino showed a higher kinetic aptitude, confirming the findings of others authors (Rauw *et al*., 1998) saying that slow-growing animals unselected for high productive performance show higher kinetic aptitude than those selected for production efficiency. At older ages, static activities are the most represented in both genotypes and rearing systems. These results are in agreement with Gunn-Dore and Morton (1993), who showed that maintenance activities have a high diurnal distribution. In young rabbits the higher static activities were performed by caged animals; at older ages NZW caged rabbits showed the highest percentage of these behaviours, 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). More comfort behaviours were observed in young animals housed in cages, a result that could represent an expression of boredom and frustration due to a lack of environmental stimuli (Gunn-Dore, 1994). At older ages, this value increased in Leprino caged rabbits. Leprino (at both ages) showed a greater expression of socials behaviours, both in young, pasture-reared rabbits and in caged older ones. Escape-alert intents were observed more frequently in pasture housed young animals; at older ages these behaviours were evident only in Leprino. The tonic immobility test showed significant differences only at the older age; Leprino showed a higher reactivity when reared in pasture while NZW reacted faster when reared in cages. The discomfort of Leprino in cages was confirmed by their mortality rate: highest in Leprino caged (30 vs. 15%, respectively for cage and pasture housing systems) and lowest for NZW in pasture (15 vs. 10%, respectively for pasture and cage housing systems).

## CONCLUSIONS

The results of this study are a preliminary reference value for intensively- and pasture-reared rabbits. However, a better understanding of the dynamics and interaction among immune traits is needed. Thus, a larger study (i.e. using pathogenfree and experimentally infected rabbits) is needed to evaluate how these parameters change and what is their relationship during the first phase of infection/inflammation. In general, the two genotypes seem to have different *in vivo* immune and oxidative patterns that may affect also the *post-mortem* evolution of meat (Mugnai *et al*., 2008). Regarding behavioural traits the results from the stereotypies displayed by Leporino rabbits confirmed their uncomfortable conditions when living in cages.

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