PASTURE AVAILABILITY AND GENOTYPE EFFECTS IN RABBITS: 2. DEVELOPMENT OF GASTRO-INTESTINAL TRACT AND IMMUNE FUNCTION OF THE VERMIPHORM APPENDIX

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ABSTRACT

The aim of this trial was to investigate the effect of pasture availability on gastro-intestinal tract and lymphoid follicle development from the vermiphorm appendix (VA) of weaned rabbits of the Leprino of Viterbo and New Zealand White (NZW) breeds. Weaned Leprino of Viterbo and NZW weaned rabbits, (30 days old; 40 animals/breed) were divided into two groups, placed into bicellular cages (17 rabbits/m^2) or wired pens (10 rabbits/m^2) and provided with an external grass pasture (1 rabbit/20 m^2). Rabbits were slaughtered at 90 days of age and body weights were recorded. The gastro-intestinal tracts were excised and weighed. Length, diameter and weight of VA were recorded as well as the number and area of the lymphoid follicles. Differences in pasture availability and locomotive activity were associated with different slaughter weights. Rabbits reared with pasture available exhibited a higher gastro-intestinal tract weight ($P<0.05$), in particular NZW rabbits which showed the greatest pasture intake. As expected, the highest weight of full gastro-intestinal tract ($P<0.05$) was observed in the two breeds with pasture availability. A slight increase in the number of lymphoid follicles in the VA from animals reared with pasture could explain a better health condition, even taking into account that this structure represents only a part of the total lymphoid tissue associated with the gut.

Key words: Rabbit, Pasture, Immune function, Vermiphorm appendix.

INTRODUCTION

The ideas of animal welfare specialists and farmers often are in conflict, as the first group prefers the large-group housing of rabbits on deep litter, while the second one prefers fattening cages in which the economical aspects are emphasized (Orova *et al*., 2004). EC Committee has established welfare rules, in particular the use of higher cages with wider floors the behaviours of the animals (Dal Bosco *et al*., 2003). The trend is to substitute the individual or bicellular cages used in some countries (e.g. Italy) with group cages or pens that allow social interactions and to increase the space available per animal that enable the expression of natural behaviors (Trocino *et al*., 2004). Growing rabbits also show a greater preference for wire net rather than straw bedded floors which soil their fur and facilitate disease transmission (Dal Bosco *et al*., 2002). Regarding the breed, it is advisable to utilize only pure breeds and their crosses of first generation and to avoid the red-eyes breeds (Dalle Zotte *et al*., 2005). The choice of breed, mainly in pens, must consider its resistance to disease, rusticity and growth rate.

Maturity of the gastro-intestinal tract mainly depends on the early intake of solid food, the intestinal enzyme activity and caecal function; rabbits at weaning have not yet adequately developed these traits (Gidenne and Fortun-Lamothe, 2002). In the gut, immune responses are initiated in the inductive sites which include Payer's patch and the Vermiphorm Appendix (VA). These are additional structures having a very important role in the digestive immune system and they contain several hundreds of dome-follicles with a special organization in adult animals (Fortun-Lamothe and Boullier, 2004). After birth, these tissues develop from non-organized immune regions into several hundred follicles that

function as an interface between food and microbial flora, reaching maximum size by 6-weeks of age (Weinstein *et al*., 1994).

The objective of this trial was to investigate the effect of pasture availability on gastro-intestinal tract and the GALT (Gut Associated Lymphoid Tissue) development on weaned rabbits of the Leprino of Viterbo and NZW breeds.

MATERIALS AND METHODS

Animals and experimental design

The trial was carried out on the experimental farm of the Department of Applied Biology (University of Perugia, Italy). Eighty weaned (35 d) Leprino of Viterbo and NZW rabbits were divided in two homogeneous groups (sex, weight and genotype) and assigned to two different housing systems:
- bicellular cages (17 rabbits/m^2) under standard fattening conditions;
- wire pens (10 rabbits/m^2), with free access to an external grass paddock (1 rabbit/20 m^2), after a week of gradual adaptation.

Feeding

Rabbits were fed *ad libitum* an organic diet, bought from a national agency. No medical treatment was provided. The composition of feed was: crude protein 16%, crude fibre 13%, fat 3% and digestible energy 11,0 MJ/kg.

Determinations

Handling and dissection of the carcasses were performed as proposed by Blasco and Ouhayoun (1996). The gastro-intestinal tract was excised and weighted. 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). In the Departamento de Producción Animal de la Universidad Politécnica de Madrid all samples were gradually dehydrated with increasing concentrations of ethyl alcohol (50-100%). These dehydrated specimens were embedded in paraffin, sectioned (6 μm) and stained with haematoxylin and eosin.

The histological sections were studied. Medial cross sections of the VA were magnified at 40× using an Olympus BX-40 light microscope and photographed for later analysis with a camera attached to a computer for image processing (P: Soft software, version 3.2 C4040Z, Olympus, Soft Imaging System GmbH, Germany). The number, perimeter, and area of the lymphoid follicles were determined only in those with complete germinal centre and dome (Castellini *et al*., 2007).

Statistical Analysis

Statistical analysis was done with a General Linear Model (GLM/SAS, 1990) with the effect of housing or pasture availability as the main sources of variation. All means were compared using the protected LSD test.

RESULTS AND DISCUSSION

Both genotypes reared in cages showed better performance in terms of slaughter weight (Table 1), perhaps because the time devoted to eating increased as a consequence of the reduced movement. Moreover, presumably, growth requirements are not adequately achieved by voluntary ingestion in alternative systems, due to the time spent in activities other than feeding (Mugnai *et al*., 2008; Hernández and Gondret, 2006). Some authors found lower slaughtering weights when studying the

effects of exercise on growth (Combes *et al*., 2005) or when comparing conventional and organic rabbits (Combes *et al*., 2003). Rearing rabbits in bicellular cages gives an advantage to breeders: their higher productivity and less contamination among animals result in a lower level of infection, disease and mortality and avoid the risk of aggressive behaviour (Szendro and Luzi, 2006). The rabbits reared with pasture available exhibited a higher gastro-intestinal tract weight (% LW; $P<0.05$), in particular the NZW breed, which showed a greater pasture intake (Mugnai *et al*., 2008). Dalle Zotte *et al*. (2005), analyzing the performances of organic rabbit production, obtained similar values in Vienna Blue and Burgundy Fawn breeds.

Table 1: Effect of pasture availability on net body and gastro-intestinal tract weight in two different breeds

		Leprino		NZW		P
		Control	Pasture	Control	Pasture	
Slaughtering weight	(g)	2563^{b}	2418^{a}	2902^{d}	2650^{c}	154
Full gastro-intestinal tract weight	(g)	462.62^{a}	458.94^{a}	521.78^{b}	493.96^{ab}	43.34
Full gastro-intestinal tract weight	(% LW)	18.05^{a}	18.98^{b}	17.98^{a}	18.64^{b}	3.93

N=40 (per group); a..d.: $P<0.05$

The morphological organization of lymphoid follicles in the rabbit VA (Table 2) was similar to that described by other authors (Dasso *et al*., 2000; Marchetti *et al*., 2004). Most lymphoid changes occur within the first 12 weeks of age in a healthy rabbit appendix (Dasso *et al*., 2000), where it is normal to observe a change in the shape and a decrease in the number of the follicles. In previous descriptions of morpho-functional parameters of GALT from pups feeding a control diet or fresh chicory (Castellini *et al*., 2007), similar difficulties in making comparisons and inferences were observed.

Table 2: Vermiphorm Appendix and follicle characteristics

		Leprino			NZW		
		Control	Pasture	SEM	Control	Pasture	SEM
VA							
Weight	(% LW)	0.31	0.26	0.08	0.38	0.38	0.09
Diameter	(mm)	1.52	1.37	0.16	1.72	1.66	0.31
Length	(mm)	11.98	11.60	1.10	12.48	12.06	1.32
Follicles							
Number		28.40	31.00	1.97	37.16	39.00	2.06
Area	(mm^2)	1.20	0.77	0.50	1.00	0.99	0.03

N=20 per genotype

Although the mucosal immunity of neonatal rabbits largely depends on the VA (Dasso and Howell, 1997), this study takes into account only a part of the total body immune response. GALT is liable for mucosal defense and regulation of inflammatory answer and can distinguish the antigens of diets and develop a mechanism to tolerate and to avoid allergies (Carabaño *et al*., 2005). In fact, Stepànkova and Kovaru (1978) observed an abnormal GALT development and a lower number of lymphocytes in animals bred in the absence of bacteria. Our result showed slight non-significant differences, in the number ($P<0.09$) and area ($P>0.05$) of follicles of the medial section of appendix when comparing rabbits that have had or not pasture available. The former probably had a higher contact with antigenic substances; in fact, for a GALT normal development the presence of food antigens is necessary, as well as flora microbial stimuli (Lanning *et al*., 2000). Pasture availability provides a better health condition in relation to the space available and the locomotive activity, exercising a positive influence in the control of digestive function (D'Agata *et al*., 2007) and presumably on the digestive response immune.

CONCLUSIONS

An effective use of pasture or a higher ingestion needed for body exercise could explain the higher weight of the full gastro-intestinal tract in animals reared with pasture available. Even in the NZW

breed, pasture availability was a profitable condition taking into account that this is a high performance selected breed.

A light increase in the number of lymphoid follicles could explain a better health condition, even taking into account that it is only a part of GALT. In the VA newly generated B cells also undergo selection processes involving self and foreign antigens and superantigens (Pospisil *et al*., 1998). Further analysis of IgG production and others indexes of specific/aspecific immunity are needed to clarify this complex phenomenon.

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