IMMUNE RESPONSE INDUCED BY FEED IN GROWING RABBITS

CANO J. L.[1], BLAS E.[2], SOLER M. D.[1], MOYA V. J.[2], GUILLÉN M. I.[1]

[1]Departamento de Producción Animal y Ciencia y Tecnología de los Alimentos.
Universidad Cardenal Herrera-CEU. 46113 Moncada. Spain.
[2]Departamento de Ciencia Animal. Universidad Politécnica de Valencia.
46071 Valencia. Spain.
eblas@dca.upv.es.

ABSTRACT

Two litters of eight young rabbits, 21-day old and deprived of feed up till then, were used. Animals were identified and fed *ad libitum* on diet A or diet B throughout the experiment, until 60-day old; the rabbit does were fed *ad libitum* on diet N. Diets A and B were formulated in such a way that no raw material was present in both and diet N was a commercial diet. At the beginning and the end of experiment, samples of blood were taken from each young rabbit as well as from their mothers. Dot-immunoblotting technique was carried out to asses the existence of anti-feed antibodies in blood serums, using soluble fractions from the *in vitro* digestion of diets A, B and N as dietary antigens. Both rabbit does presented high reactivity against all tested diets in both initial and final samples. The serum of rabbits at 21-day old also showed high reactivity against all tested diets. At 60-day old, reactivity of serum of rabbits showed different pattern depending on the diet they consumed. In the most of cases of animals consuming diet A, clear decrease of levels of antibodies against diets B and N but increase or maintenance of levels of antibodies against diet A were observed. In the most of cases of animals consuming diet B, decrease of levels of antibodies against diets A and N was detected together with decrease or maintenance of levels of antibodies against diet B. These results point to the existence of anti-feed IgG antibodies in blood of adult rabbit does, transferred to litter, as well as to a specific immune response of rabbits to the feed that they consumed around weaning of variable intensity depending on the feed composition.

Key words: immune response, anti-feed antibodies, dot-immunoblotting.

INTRODUCTION

The immune system is fitted to react against molecules strange to organism, which are not recognised as own and producing a defensive response. Feeds are very complex mixture of organic molecules initially strange to organism and then with potential capability to induce immune responses. In the digestive tract, breakdown of macromolecules and absorption of nutrients occurs but also pathogen and harmful

agents must be identified and neutralized by immune system to avoid their penetration through intestinal way. To carry out these two functions, in some way antagonistic, the immune system have several complex mechanisms to regulate the local and systemic immune response in order to avoid adverse reactions to feed, what would mean important problems of health and survival of individuals (TIZARD, 2000).

An initial mechanism to avoid the induction of immune reactions by feed is the digestive process itself. Digestion means deep hydrolysis of complex macromolecules to single molecules to be absorbed by intestinal mucosa. These molecules resulting from digestive process have not capacity to stimulate the immune system because of their low molecular weight (HEYMAN, 2001). However, this protective mechanism could be strongly challenged in young rabbits around weaning, especially if early, when they are not still able to digest feeds efficiently. Thus, dietary large-size molecules, potentially antigenic, would arrive to intestinal mucosa and trigger strong immune reactions (KELLY AND COUTTS, 2000). These phenomena of immune response to dietary antigens are involved in intestinal troubles and low performance in piglets (LI *et al*., 1991) and pre-ruminants (LALLES *et al.*, 1998). Similar problems would be originated when feed supplied to young rabbits is not designed in accordance with their digestive degree of maturing, as usually occurs in intensive rabbit production.

To initial assessment of this potential immune-nutritional risk in growing rabbits, it seems interesting to study if massive arrival of dietary antigens to intestinal mucosa can induce some immune response being revealed by presence in blood of specific IgG antibodies against feed that they are consuming.

MATERIAL AND METHODS

Animals

Two litters of eight young rabbits, 21-day old and deprived of feed up till then (by caging separately of their mothers but suckling once a day until weaning at 28-day old), were used. Animals were identified and fed *ad libitum* on diet A (litter of rabbit doe number 56) or diet B (litter of rabbit doe number 71) throughout the experiment, until 60-day old; the rabbit does were fed *ad libitum* on diet N. At the beginning and the end of experiment, samples of blood were taken from each young rabbit as well as from the mothers, by puncture in the central artery of ear. Three young rabbits died during the experiment.

Diets

Diets A and B were formulated in such a way that no raw material was present in both, in order to obtain proteins from separate origins, and thus high antigenic divergence (Table 1). Diet N was a commercial diet.

Table 1. Ingredients of diets A and B (%).

	Diet	
	A	B
Beet pulp	40	
Soybean meal	20	
Soybean hulls	20	
Straw	10	
Soybean oil	7	
Wheat		18.5
Wheat bran		18.5
Alfalfa		55
Fish meal		5
Lysine	0.3	0.3
Methionine	0.1	0.1
Treonine	0.1	0.1
Calcium carbonate	0.2	0.2
Calcium hydrogen phosphate	1.2	1.2
Sodium chloride	0.6	0.6
Trace element-vitamin mixture	0.5	0.5

Dot-immunoblotting

Dot-immunoblotting technique was carried out to asses the existence of anti-feed antibodies in blood serums. As dietary antigens, soluble fractions from the *in vitro* digestion of each diet according PASCUAL *et al*. (2000), as well as from *in vitro* digestion without any feed (control), were used. Dietary antigens were adsorbed to nitrocellulose membrane according the following quadrant arrangement: left-upper: diet A; right-upper: diet B; left-lower: diet N; right-lower: control. As many membranes as serum to test were prepared. Membranes were blocked with skimmed milk (3% in 20 mM PBS pH 7.0 - 0.1% Tween-20) during 30' at room temperature and afterwards each one was incubated during two hours at 37 ºC with a 1/10 dilution of one of the serums to test. After thorough washing with PBS-Tween, membranes were incubated with goat anti-rabbit IgG antibody labelled with horseradish peroxidase. The resulting immune complex was revealed by chemioluminiscency on X-ray film, where intensities of antigen-antibody reaction areas are indicative of the amount of IgG antibodies linked to dietary antigens.

RESULTS AND DISCUSSION

Figures 1 and 2 show the intensities of antigen-antibody reaction areas from serums of litters feeding respectively diet A or B, as well as from their own mothers. Both rabbit does presented high reactivity against all tested diets in both initial and final samples, it indicating presence of antibodies reacting with a great variety of dietary antigens. That could be explained because these adult animals were always fed on commercial feeds, formulated with a great variety of raw materials and producing multiple antigenic

stimulation. The reactions against the control, less intense and not present in all tested samples, could indicate the presence of antibodies recognising epitopes of bovine pepsin used in the *in vitro* digestion technique, these antibodies being induced by other antigens that would produce these less specific crossed reactions.

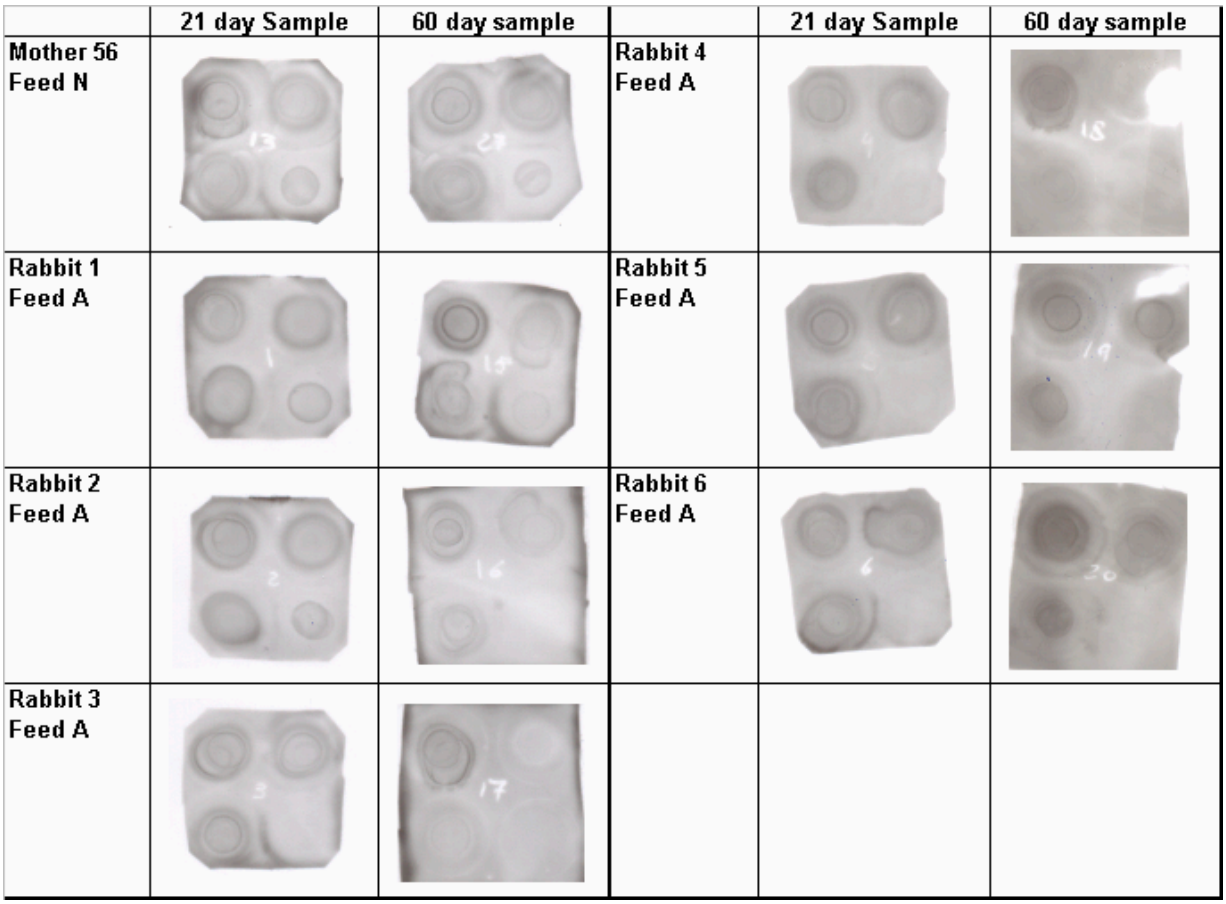

Figure 1. Dot-immunoblotings of serums from rabbits feeding diet A and from their mother (fed on diet N).

The serum of rabbits at 21-day old also showed high reactivity against all tested diets, originating as similar pattern pictures as produced by serums of the rabbit does. Taking into account the limited immune response in very young animals and that these animals were exclusively suckling and had not consumed any feed up till then, these results could be caused by a maternal transmission of anti-feed antibodies.

At 60-day old, reactivity of serum of rabbits showed different pattern depending on the diet they consumed. In the most of cases of animals consuming diet A, clear decrease of levels of antibodies against diets B and N but increase or maintenance of levels of antibodies against diet A were observed. In the most of cases of animals consuming diet B, decrease of levels of antibodies against diets A and N was detected together with decrease or maintenance of levels of antibodies against diet B. These results could indicate the induction of a specific immune response to feed in young rabbits fed on diet

A, not so evident in the case of young rabbits fed on diet B. A possible difference in the level of antigenicity among diets A and B could be related with the high level of inclusion of soybean meal in diet A and its absence in diet B, because of the well know antigenicity of this raw material (CHRISTENSEN *et al*., 2003); additionally, feed intake was much lower in diet A than with diet B (43 *vs* 75 g/young rabbit and day between 21 and 49-day old). The decrease of antibodies against the feeds that animals had not consumed indicates a lack of antigenic stimulus and is congruent with the kinetics of disappearance of transferred maternal antibodies.

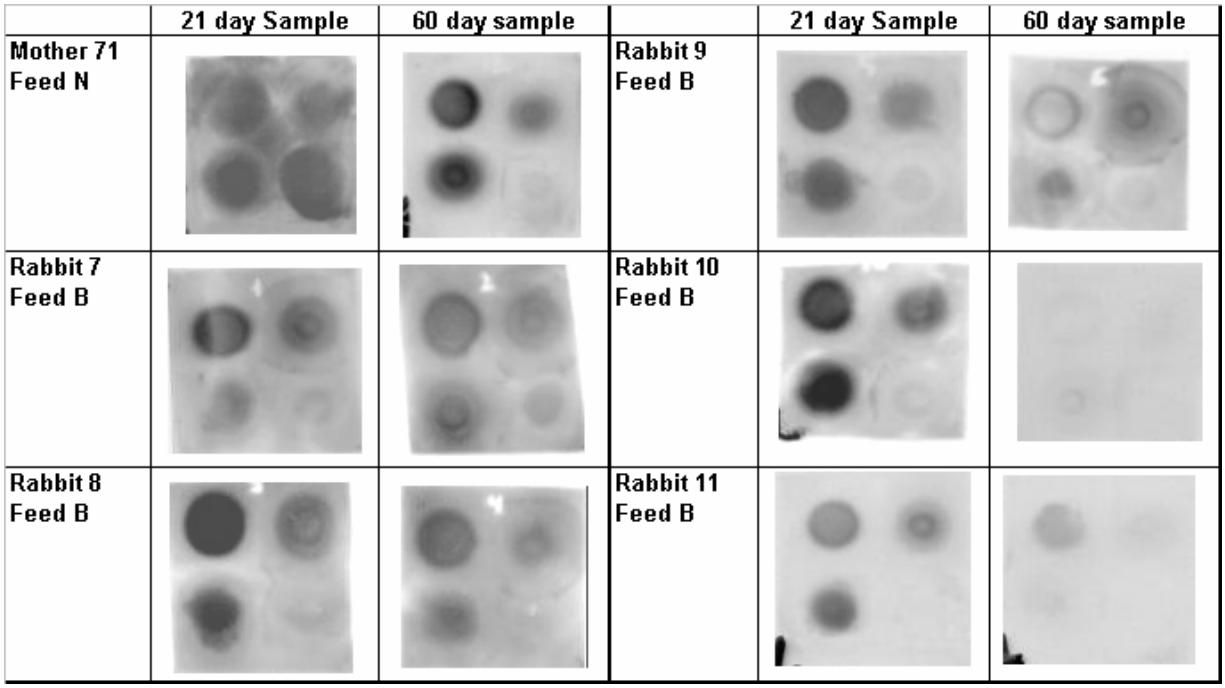

Figure 2. Dot-immunoblotings of serums from rabbits feeding diet B and from their mother (fed on diet N).

CONCLUSIONS

These results point to the existence of anti-feed IgG antibodies in blood of adult rabbit does, transferred to litter, as well as to a specific immune response of rabbits to the feed that they consumed around weaning of variable intensity depending on the feed composition. Further work is necessary to asses the significance of the immuno-nutritional side of feeding around weaning.

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