EFFECT OF DIETARY LEVEL AND SOURCE OF GLUTAMINE ON INTESTINAL HEALTH IN THE POSTWEANING PERIOD

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ABSTRACT

The aim of this work was to study the effect of dietary glutamine (gln) level and source on rabbit mortality rates induced by Epizootic Rabbit Enteropathy (ERE) and the detection of *C. perfringens* from intestinal isolates. The effect of this amino acid on mucosal barrier mechanisms was also assessed by studying the relative PPARγ mRNA abundance in ileum and jejunum. Three diets were experimentally tested, a basal diet (C) containing 177 g of CP/kg DM, and 28.8 g of gln/kg DM, and two diets supplemented at 0.5% and 1% above the basal levels (C-G0.5 and C-G1). In addition, to study the effects of including 1% gln as synthetic vs. a natural source, a fourth diet (W) was included in the experiment containing a wheat protein concentrate (VITEN cws). One hundred and ninety-six individually lodged animals (49 animals/treatment) and two hundred and twelve collectively lodged animals (53 cages/treatment) weighing 476±93 g and 498±100 g respectively were blocked by litter and randomly assigned to the experimental diets. Animals were fed the experimental diets for 18 d (28 to 46 d of age). During the second period (46 to 60 d of age) all rabbits were fed the basal diet (C). For the microbiological caecal study 60 rabbits with 44 d of age (15 per treatment) weighing 565±96 g were selected. For the RNA isolation and real-time RT-PCR 7 rabbits per treatment (C, C-G0.5 and C-G1) from the microbiological analysis were selected. The supplementation with 0.5 L-gln resulted in a reduction of 1.1 log unit of *C. perfringens* CFU/g (P=0.05) compared to the basal diet. Also a tendency for a quadratic effect of dietary gln level (P=0.09) was observed. Higher relative abundance of PPARγ mRNA was observed in ileum than in jejunum, however, no dietary effects were reported either in ileum or in jejunum. No differences were observed between sick and healthy animals. Our results suggest glutamine plays an important role in the intestinal health without effects on growth performances. However, gln level had no effect on PPARγ mRNA abundance partly explained by the lack of a clear inflammatory process in sick animals. Optimal results were obtained with 0.5% of synthetic gln supplementation. No differences between the two sources of gln were detected for any of the traits studied. Therefore, synthetic gln sources might be substituted by natural ones, as wheat protein concentrate, decreasing feeding costs.

Key words: Rabbit, ERE, Mortality, Glutamine, PPARγ.

INTRODUCTION

Some amino acids as glutamine (gln), which acts as an indispensable metabolic fuel to maintain mucosa functionality in humans (Brooks *et al*., 1998), seems to offer good results in rabbits. Chamorro *et al*., (2007a,b) indicate that dietary supplementation with 1% of gln reduces the mortality caused by Epizootic Rabbit Enteropathy (ERE), modifies ileal microbiota, (with a decrease of the frequency of detection of several pathogens as *C. perfringens* and *Helicobacter* spp), and diminishes the presence of *Eimeria* at the jejunum. Although, these results suggest that gln improves the mucosa barrier mechanisms they were unable to see any effect on villous length or in the N-amino peptidase activity. This effect on mucosal morphology is opposite to that observed in pigs (Wu *et al*., 1996), suggesting a different mode of action in rabbits. Yeh *et al*. (2005) reported a decrease on pro-inflammatory citoquine production after dietary gln supplementation pointing to a relevant role of this amino acid for a correct enterocyte response during catabolic, inflammatory and infectious processes. The

incidence of inflammatory processes may be associated in rabbits affected by ERE (Licois *et al*., 2005), although Marlier *et al.* (2006) were unable to observe intestinal inflammation after a gross lesion analysis.The aim of this work was to study the effect of dietary gln source and level on rabbit mortality rate the detection of *C. perfringens* from intestinal isolates, and on mucosal barrier mechanisms by studying the effect of dietary gln level on PPARγ expression.

MATERIALS AND METHODS

Experimental Diets. To determinate the effects of dietary gln level, three diets (Table 1) were experimentally tested, a basal diet (C) containing 177 g of CP/kg DM, and 28.8 g of gln/kg DM, and two diets supplemented at 0.5% and 1% above the basal levels (basal + 0.5% L-gln; C-G0.5 and basal + 1% L-gln; C-G1). In addition, to study the effects of including 1% gln as synthetic vs. a natural source a fourth diet (W) was included in the experiment containing a wheat protein concentrate (VITEN cws 752 g/kg DM basis of CP and 29.2 g/kg DM of gln).

Animals and experimental design. All the experiments were carried out with New Zealand White x Californian rabbits from a farm affected by ERE. Animals were housed in both individual and collective (2 rabbits/cage) flat-deck cages. Rabbits were weaned at 28 days of age. Temperature conditions were kept between 15 and 25ºC by heating and cooling systems combined with continuous forced ventilation.

Mortality Trial. One hundred and ninety-six individually lodged animals (49 animals/treatment) and two hundred and twelve collectively lodged animals (53 animals/treatment) weighing 476±93 g and 498±100 g respectively were blocked by litter and randomly assigned to the experimental diets. Animals were fed the experimental diets for 18 d (28 to 46 d of age). During the second period (46 to 60 d of age) all rabbits were fed only with the basal diet (C). Feed and water was offered *ad libitum*. Not antibiotics were used. Mortality was controlled daily during the whole experiment.

Chemical and Microbiological Analyses. Procedures of the AOAC (2000) were used to determine the dietary concentrations of DM (934.01), ash (967.05), CP (968.06), ether extract (920.39), and starch (amyloglucosidase-α-amylase method, 996.11). Dietary NDF, ADF and ADL were determined sequentially by using the filter bag system (Ankom Technology, New York) according to Mertens (2002), and AOAC (2000; procedure 973.187). For the microbiological caecal study sixty collectively lodged rabbits (15/treatment) with 44 days of age weighing 565±96 g, were selected. Within each treatment, care was taken to select both normal and displaying visual symptoms of ERE (such as diarrhoea and mucus secretion) animals. Samples were analyzed following the ISO 7937, 1997.

RNA isolation and real-time RT-PCR.For RNA isolation, 7 rabbits per treatment (C, C-G0.5 and C-G1) from the microbiological trial were selected. Segments of 3 cm from the middle part of ileum and jejunum were removed immediately after euthanasia, cleaned with physiological salt buffers (0.9% NaCl), snap frozen in liquid nitrogen and stored -80°C until total RNA extraction. Total RNA isolation from ileal and jejunum mucosal scrappings, cDNA synthesis, and PPARγ-mRNA relative abundance was assessed as described in Diez *et al*. (2007). Primers and probes for rabbit PPARγ (Genbank access. no. AY166780) and the reference gene (GAPDH; Genbank access. no. AB231852) were designed using Primer Express® v.2 (Applied Biosystems, Foster City, CA, USA). All samples were run in triplicate and quantified by normalizing the PPAR signal to that of GADPH by the "Delta-Method" (Comparative Ct Method, ABI Prism 7700 user Bulletin #2).

Table 1: Ingredients and chemical composition of the experimental diets[1]

	C1	C-G0.5	C-G1	W
Ingredients (% DM):				
Soft Wheat	25	-	-	25
Wheat bran	26.5	-	-	25.35
Sunflower meal	8	-	-	6
Alfalfa hay	35	-	-	30
Straw	2	-	-	6.5
Lard	2	-	-	2
L-Lysine HCL	0.2	-	-	0.25
DL- Methionine	0.15	-	-	0.15
L-Threonine	0.15	-	-	0.2
L-glutamine	0	0.5	1	0
Sodium chloride	0.5	-	-	0.5
Vitamin/mineral premix[2]	0.5	-	-	0.5
VITEN cws[3]	0	-	-	3.55
Analyzed composition (g/kg DM):				
DM	91.4	91.5	91.5	91.7
CP	177	182	188	192
Starch	259	257	258	254
NDF	338	333	335	337
ADF	190	188	189	185
ADL	55	54	54	5
Calculated composition[4,] (g/kg DM)				
Lysine	8.5	8.4	8.4	8.2
Methionine	4.4	4.4	4.4	4.4
Methionine + cystine	7.4	7.4	7.4	7.4
Threonine	7.6	7.6	7.6	7.5
Glutamine	28.8	33.4	38.8	37.9

[1]Basal diet (C); basal + 0.5% L-glutamine (C-G0.5); basal + 1% L- glutamine; 1% of Glutamine added with a Wheat protein concentrate diet (W).[2] Provided by Trouw Nutrition International.[3] Wheat protein concentrate. Provided by Trouw Nutrition International. [4]Calculated values according to FEDNA (2003)

Statistical Analysis. The mortality and the *C. perfringens* logarithm CFU/g of digesta data were analyzed as a completely randomized block design with litter as block by using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC, 1998). The main effects studied were the level and source of gln. Data are presented in tables as least squares means. Squares sums of gln addition were partitioned into linear and quadratic effects. Contrasts also were used to compare the source of gln (W vs. C-G1). The effect of the treatment on microbiological results was analyzed only in sick animals (those whose *C. perfringens* CFU/g was greater than 10^5 according to Chamorro *et al.* (2007c). Also SAS was used to analyze gene expression. A General Linear Model procedure was used to determine whether a significant difference was present among the dietary gln level.

RESULTS AND DISCUSSION

As in our previous studies mortality was related to ERE (Chamorro *et al*, 2007c; Gomez Conde *et al*., 2007). Animals declared as sick showed higher counts of *C. perfringens* than those declared as healthy (6.8 vs 3.6 *C. perfringens* CFU/g logarithm on average). Most of sick animals (80%) displayed the clinical signs described for the disease (Pérez de Rozas *et al*., 2005; Licois *et al*., 2006). The maximum mortality rates were recorded in the second and third week after weaning. During the first growing period a quadratic effect of dietary gln inclusion level was observed (P=0.024) resulting in a decrease of around 47% mortality in rabbits fed the C-G0.5 diet (Table 2). A similar effect (P=0.004) was observed in the overall period. Mortality tended (P=0.093) to be lower in rabbits fed the C-G0.5 than the W diet in the whole growing period. The supplementation with 0.5 L-gln resulted in a reduction of 1.1 log unit of *C. perfringens* CFU/g (P=0.05) compared to the basal diet (Table 2). Also, a tendency for a quadratic effect of dietary gln level (P=0.09) was observed. The enhanced sanitary status observed in rabbits fed the gln supplemented diets may be due to the positive effect of this amino acid on mucosal barrier mechanisms or/and throughout changes on the intestinal microbiota.

Table 2: Effect of experimental diets on mortality and *C. perfringens*

	C[1]	C-G0.5	C-G1	W		Contrast 1[b]	2[c]	3[d]
					SEM[a]	P-value		
Mortality 28 to 46 d of age (%)	22.1	11.8	22.2	17.1	3.72	NS	0.024	NS
Mortality 28 to 60 d of age (%)	25.1	13.8	30.9	21.2	4.04	NS	0.004	0.093
Logarithm of CFU/g	7.31	6.22	6.79	7.18	0.41	NS	0.09	NS

[1] Basal diet (C); basal + 0.5% L-glutamine (C-G0.5); basal + 1% L- glutamine; 1% of Glutamine added with a Wheat protein concentrate diet (W).2 NS: non significant (P>0.05).[a]n= 151 rabbits on the mortality trial.; n= 8 rabbits on the microbiological analysis. [b]Linear effect of dietary glutamine level. [c]Quadratic effect of dietary glutamine level. [d]Effect of dietary glutamine source (W vs. C-G1)

Gln helps to maintain a healthy intestine through different mechanisms including epithelial cell proliferation and apoptosis, fuelling gut metabolism and also modulates the inflammatory response (Hubert-Buron *et al*., 2006). Moreover, gln seems to act as a direct ligand for PPARγ attenuating inflammation and injury in a rodent postischemic intestine model (Sato et al., 2006). The effect of dietary gln level on PPARγ mRNA abundance in ileum and jejunum are shown in Figure 1. Higher relative abundance of PPARγ mRNA was observed in ileum than in jejunum, however, no dietary effects were reported either in ileum or in jejunum (Figure 1A). Also, no differences were detected among normal and sick animals (Figure 1B). These results are in agreement with Chamorro *et al*. (2007a) who reported no differences in mielo-peroxidase values when both diseased and healthy animals were compared. This suggests an absence of mucosal inflammatory response as observed by Marlier *et al*. (2006). The source of gln (synthetic vs. wheat protein isolated) showed no effects on mortality or *C. perfringens* counts. The high protein ileal digestibility expected for VITEN could reduce N flow at ileal level and hence explain the lack of response in the presence of *C. perfringens* as observed by Chamorro *et al*., (2007c).

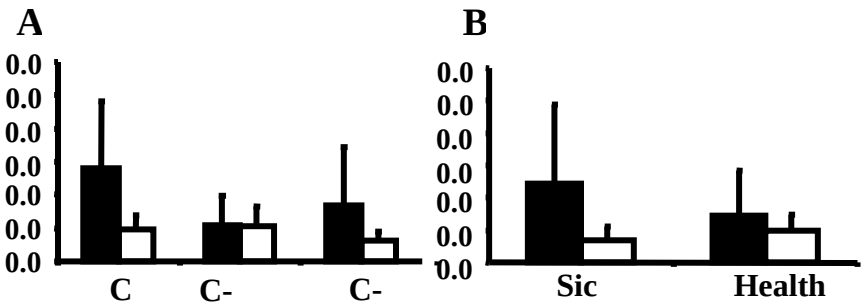

Figure 1: Effect of dietary glutamine level on relative gamma peroxisome proliferator activated receptor (PPARγ) mRNA abundance in ileum (■) and jejunum (□)

CONCLUSIONS

Glutamine plays an important role in the intestinal health without effects on growth performances. Dietary gln supplementation (0.5%) reduces the mortality caused by ERE, modifies the intestinal microbiota limiting the presence of *C. perfringens* associated with the incidence of ERE. However, dietary gln level had no effect on PPARγ mRNA abundance partly explained by the lack of a clear inflammatory process in sick animals. Under our experimental conditions synthetic gln sources might be substituted by natural ones, as wheat protein concentrate, decreasing feeding costs.

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