Effects of Dietary Glutamine Supplementation Levels on Fur Quality and Intestinal Barrier Function in Rex Rabbits from Weaning to Three Months of Age (Postprint)

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Abstract

This study aimed to investigate the effects of dietary glutamine supplementation level on fur quality and intestinal barrier function in weaned Rex rabbits from weaning to 3 months of age. A total of 180 weaned Rex rabbits with similar body weight were randomly assigned to 5 groups, with 36 replicates per group (1 rabbit per replicate). The groups were fed experimental diets containing glutamine at supplementation levels of 0 (control), 0.3%, 0.6%, 0.9%, and 1.2%, respectively. The experiment included a 7-day pre-trial period and a 56-day formal trial period. The results showed that dietary glutamine supplementation level had no significant effects on fur area, fur weight, hair length, or hair thickness in growing Rex rabbits ($P>0.05$). Dietary glutamine supplementation level significantly affected the duodenal villus height/crypt depth ratio in growing Rex rabbits ($P<0.05$). Compared with the control group, dietary supplementation with 0.9% glutamine significantly increased the mRNA expression level of zonula occludens protein in the jejunum ($P<0.05$) and significantly decreased the mRNA expression level of pyruvate kinase in the jejunum ($P<0.05$). Additionally, dietary supplementation with 0.9% glutamine significantly increased the content of secretory immunoglobulin A in duodenal mucosa ($P<0.05$). In conclusion, dietary glutamine supplementation level did not affect fur quality but improved intestinal mechanical barrier and immune barrier functions in growing Rex rabbits. Under the conditions of this experiment, the appropriate dietary glutamine supplementation level for weaned Rex rabbits from weaning to 3 months of age was 0.9%.

Full Text

Effects of Dietary Glutamine Supplemental Level on Fur Quality and Intestinal Barrier of Rex Rabbits during Weaner to 3-Month-Old

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Abstract

This experiment was conducted to investigate the effects of dietary glutamine (Gln) supplemental level on fur quality and intestinal barrier function of Rex rabbits from weaning to 3 months of age. One hundred eighty weaned Rex rabbits with similar body weight were randomly assigned to 5 groups, each consisting of 36 replicates with 1 rabbit per replicate. The five groups were fed experimental diets supplemented with 0% (control), 0.3%, 0.6%, 0.9%, and 1.2% Gln, respectively. The pre-test period lasted 7 days, followed by a 56-day experimental period.

The results showed that dietary Gln supplemental level had no significant effect on fur area, fur weight, hair length, or hair thickness of growing Rex rabbits ($P>0.05$). However, dietary Gln supplemental level significantly affected the villus height to crypt depth ratio in the duodenum ($P<0.05$). Compared with the control group, dietary supplementation with 0.9% Gln significantly increased the mRNA expression of zonula occludens-1 (ZO1) in the jejunum ($P<0.05$) while significantly decreasing the mRNA expression of pyruvate kinase (PK) in the jejunum ($P<0.05$). Additionally, dietary supplementation with 0.9% Gln significantly increased the content of secretory immunoglobulin A (sIgA) in duodenal mucosa ($P<0.05$).

In conclusion, although dietary Gln supplemental level did not affect the fur quality of growing Rex rabbits, it improved both intestinal mechanical barrier and immune barrier functions. Under the conditions of this experiment, the appropriate Gln supplemental level in the diet for Rex rabbits from weaning to 3 months of age is 0.9%.

Keywords: glutamine; growing Rex rabbits; intestinal barrier; fur quality

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Introduction

In recent years, the special nutritional functions of glutamine (Gln) in intestinal barrier and other aspects have gradually become a research hotspot. Gln provides the metabolic needs of intestinal mucosal cells and serves as a precursor for the synthesis of pyrimidine, purine nucleotides, and amino sugars. Numerous studies have found that Gln is a non-essential amino acid under healthy

conditions, but becomes essential when intestinal mucosa is damaged or Gln is depleted due to chemotherapy, starvation, radiation therapy, and other conditions. Therefore, Gln is referred to as a conditionally essential amino acid. Windmueller0 demonstrated that Gln is the primary energy donor for intestinal epithelial cell metabolism and a necessary nutrient for the proliferation of intestinal epithelial lymphoid tissue. Qin Huanlong et al.0 reported that Gln is also one of the main functional substances for macrophages and lymphocytes, and that Gln supplementation can promote the secretion of secretory immunoglobulin A (sIgA), reduce the rate of bacterial translocation, and thereby protect intestinal barrier function0. Hauf et al.0 showed that in a piglet model infected with E. coli, dietary Gln supplementation could alleviate disease severity and reduce the incidence of diarrhea. Rabbits have thin intestinal walls that are susceptible to external stimuli and various intestinal diseases, particularly during the weaning stage when they face multiple stressors including weaning, feed changes, and environmental changes, which increase intestinal burden and susceptibility to damage. Whether Gln also has a beneficial effect on intestinal barrier function in rabbits remains unknown.

Rex rabbits are a dual-purpose breed valued for both meat and fur. Fu Chaohui et al.0 investigated the effects of different dietary Gln levels on the growth and development of Rex rabbits and found that dietary Gln supplementation at 0–1.2% had no significant effect on body weight gain or feed intake. However, dietary Gln supplementation can promote the growth and development of pigs00 and broilers00, indicating significant species differences in Gln requirements. The economic value of Rex rabbits is determined by their fur quality, which is affected by season, age, and nutritional level. Previous research has established the effects of dietary protein level and certain amino acids (methionine, lysine, cysteine, and tryptophan) on fur quality in Rex rabbits. Whether dietary Gln supplementation can also improve fur quality in Rex rabbits requires further investigation. Nutritional level is an important factor affecting fur quality, and the intestine is the primary site for nutrient absorption. Therefore, intestinal health status inevitably affects fur quality in Rex rabbits, and Gln may influence fur development by regulating intestinal health. This experiment was conducted to determine the appropriate dietary Gln supplemental level for Rex rabbits by measuring fur quality and intestinal barrier indicators, thereby providing a theoretical basis for Gln application in Rex rabbit diets.

Experimental Animals and Management

One hundred eighty 30-day-old weaned Rex rabbits with similar body weight [(1,050±30) g] were selected and randomly divided into 5 groups, with 36 replicates per group and 1 rabbit per replicate. The five groups were fed experimental diets supplemented with 0% (control), 0.3%, 0.6%, 0.9%, and 1.2% Gln, respectively. The Gln used was L-Gln provided by Beijing Jia Kang Yuan Company with a purity of 99%. The basal diet was formulated according to the rabbit feeding standards of De Blas et al.0. The composition and nutrient levels of

the basal diet are shown in Table 1 . The experimental rabbits were managed under conventional feeding and immunization programs with natural lighting and ventilation, and free access to water. The rabbit house was disinfected once every 3–5 days. The pre-test period lasted 7 days, and the experimental period lasted 56 days.

Sample Collection and Processing

At the end of the experiment, 8 rabbits were randomly selected from each group. Blood samples were immediately collected via cardiac puncture, placed in a 37°C water bath for 40 minutes, then centrifuged at 3,000 r/min for 15 minutes to separate serum, which was stored at -20°C. After euthanasia by cervical dislocation, samples of duodenum, jejunum, and ileum were collected in duplicate. One portion was washed with cooled phosphate buffer saline (PBS, pH 7.4) and stored in 10% freshly prepared cooled formaldehyde solution for histological examination. The other portion was washed with PBS to remove intestinal contents, and the mucosa was carefully scraped with sterile glass slides, aliquoted into multiple EP tubes, snap-frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

Total RNA Extraction and Real-time Fluorescent Quantitative PCR

Total RNA was extracted from intestinal tissues using the Trizol method. The extracted RNA was reverse-transcribed into cDNA using kits provided by Takara (Dalian Bao Biological Co., Ltd.). Real-time fluorescent quantitative PCR was performed using the SYBR Green I method with a kit from Dalian Bioengineering (DRR041A) on an ABI7500 fluorescent quantitative PCR instrument (USA). Primer sequences are shown in Table 2 . Data were analyzed using the $2-\Delta\Delta CT$ method, and target gene mRNA expression was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA.

sIgA Content Detection

The content of sIgA was measured using a validated enzyme-linked immunosorbent assay kit containing sIgA-specific antibodies (Wuhan USCN Science Co., Ltd.). The coefficient of variation between sIgA measurements was 2.7%.

Histological Examination

Tissue samples were processed through washing, gradient alcohol dehydration, xylene clearing, and paraffin embedding, then sectioned at 5 m thickness and stained with routine hematoxylin-eosin (HE). The small intestinal mucosal villi were observed using a microscope imaging system. Five fields of view were selected per section, with five villi measured per field. Villus height (VH) and crypt depth (CD) were measured using Image-Plus software, and the villus height to crypt depth (V/C) ratio was calculated. Villus height was defined as the distance from the intestinal gland-villus junction to the villus tip, while

crypt depth was defined as the distance from the intestinal gland-villus junction to the base of the intestinal gland.

Fur Quality Determination

Fur weight (g): After slaughter, the complete fur was obtained by making circular cuts at the neck, front limb joints, and hind limb joints, then cutting along the inner sides of both hind limbs from the upper pubic region. The skin was peeled off by turning it inside out in a retreating manner. The Rex rabbit skin was weighed on a sensitive balance.

Fur area (cm^2): The obtained Rex rabbit skin was cut along the midline, laid flat in a tray in a naturally stretched state. Length was measured from the neck to the base of the tail, while width was measured at the middle of the waist (generally the widest part). The fur area was calculated by multiplying the measured length and width.

Hair length (cm): A ruler was inserted into the hair coat, and hair length was measured from the root to the tip. Each rabbit was measured at fixed positions on the shoulder, thigh, and back, and the average of the three measurements was calculated.

Hair thickness (cm): The thickness of the skin was measured at fixed positions on the shoulder, thigh, and back of each experimental rabbit using a vernier caliper, and the average value was taken.

Statistical Analysis

Experimental data were analyzed using the ANOVA procedure in SAS 8.0 statistical software for one-way analysis of variance. If significant differences among groups were detected, Duncan' s multiple comparison test was used. Data were expressed as means ± root mean square error (R-MSE). $P<0.05$ was considered statistically significant, and 0.05 $P<0.10$ was considered a significant trend.

Effects of Dietary Gln Supplemental Level on Fur Quality of Growing Rex Rabbits

As shown in Table 3 , dietary Gln supplemental level had no significant effect on fur area, fur weight, hair length, or hair thickness of growing Rex rabbits ($P>0.05$).

Effects of Dietary Gln Supplemental Level on Intestinal Mechanical Barrier of Growing Rex Rabbits

As shown in Table 4 , dietary Gln supplemental level significantly affected the villus height to crypt depth ratio in the duodenum of growing Rex rabbits ($P<0.05$), but had no significant effect on villus height or crypt depth in the duodenum ($P>0.05$). Dietary Gln supplemental level had no significant effect

on any parameters (villus height, crypt depth, villus height to crypt depth ratio) in the jejunum or ileum (P>0.05).

As shown in Figure 1 [Figure 1: see original paper], dietary Gln supplemental level significantly affected the mRNA expression of zonula occludens-1 (ZO1) and pyruvate kinase (PK) in the intestine of growing Rex rabbits (P<0.05). Compared with the control group, dietary supplementation with 0.9% Gln significantly increased ZO1 mRNA expression in the intestine while significantly decreasing PK mRNA expression (P<0.05). Additionally, dietary Gln supplemental level had no significant effect on the mRNA expression of mammalian target of rapamycin (mTOR) in the intestine of growing Rex rabbits (P>0.05).

Effects of Dietary Gln Supplemental Level on Intestinal Mucosal sIgA Content of Growing Rex Rabbits

Dietary Gln supplemental level had no significant effect on sIgA content in the ileum of growing Rex rabbits (P>0.05), but significantly affected sIgA content in the duodenum (P<0.05). When dietary Gln supplemental level was 0.9%, duodenal sIgA content reached its maximum value, which was significantly higher than the other four groups (P<0.05). Dietary Gln supplemental level showed a trend to increase sIgA content in the jejunum (0.05 P<0.10), reaching its maximum value at 0.9% Gln supplementation.

Discussion

Effects of Dietary Gln Supplemental Level on Fur Quality of Growing Rex Rabbits Fur area directly determines the use value of Rex rabbit pelts, with fur quality, skin texture, and area being the main indicators for evaluating pelt quality. Previous studies have found that hair follicle cells can directly utilize Gln and glucose for oxidative energy supply0, and therefore Gln can promote hair follicle development. In clinical practice, there are also cases of direct Gln administration for treating hair loss. However, in the current study, dietary Gln supplementation did not significantly improve fur quality in Rex rabbits. We speculate that this may be due to the relatively low Gln supplemental level. During the post-weaning period, Rex rabbits experience severe stress responses, resulting in increased Gln requirements. The current supplemental levels may only be sufficient to meet the needs of intestinal cell development and recovery, with minimal impact on hair follicle cells.

Effects of Dietary Gln Supplemental Level on Intestinal Mechanical Barrier of Growing Rex Rabbits The intestinal mechanical barrier, as an important component of intestinal mucosal barrier function, represents the most critical defense line of the intestinal defense system. It is composed of intestinal mucosal epithelial cells, intercellular tight junctions, submucosal lamina propria, and bacterial biofilm0. Studies have shown that Gln affects intestinal mucosal development, with appropriate Gln levels inducing intestinal mucosal growth, increasing small intestinal villus height, and deepening crypts in rats

and piglets0. In this experiment, dietary Gln supplementation at 0.3%–1.2% significantly increased the villus height to crypt depth ratio in the duodenum of weaned to 3-month-old Rex rabbits, indicating that Gln supplementation promotes the proliferation and differentiation of intestinal mucosal cells, facilitates repair of rabbit intestinal epithelial cells, prevents villus atrophy, reduces intestinal mucosal damage, and thereby maintains normal small intestinal mucosal morphology0.

Furthermore, energy supply is fundamental for intestinal cell function. PK is an important regulatory enzyme for glucose metabolism. Dietary supplementation with 0.9% Gln reduced PK mRNA expression in the jejunum, reflecting that Gln affects intestinal energy metabolism. The possible reason is that rapidly dividing cells such as intestinal cells can also utilize Gln for energy supply. With adequate Gln provided in the diet, the glucose metabolic pathway is somewhat inhibited, but intestinal cells can use Gln as an energy source, thereby ensuring normal intestinal physiological function0. mTOR is another energy sensor that regulates intracellular amino acid and energy metabolism. Nicklin et al.0 reported that Gln uptake and its interaction with other essential amino acids in cells is the limiting step for activating the mTOR system, and elevated dietary Gln levels provide a switch for essential amino acid uptake. However, in this experiment, dietary Gln supplemental level did not significantly affect mTOR mRNA expression in rabbit intestine. This finding is inconsistent with studies in pigs, where Xiao Yingping et al. error! reference source not found. found that dietary Gln supplementation significantly increased mTOR mRNA expression in the jejunum of weaned piglets. These results reveal that the effect of Gln on intestinal mTOR is species-dependent.

Intestinal mucosal permeability to macromolecules is an important indicator for evaluating small intestinal mucosal barrier function. Gln deficiency can cause disruption of tight junction structures between rat intestinal epithelial cells. Cui Wei et al.0 further demonstrated in experiments with human colon cancer Caco-2 cells that Gln deficiency increases intestinal epithelial cell barrier permeability, while Gln supplementation can block these changes. ZO1 is the fundamental structural protein of intestinal mucosal tight junctions, primarily functioning to connect transmembrane proteins with the cytoskeleton and transmit signaling molecules, regulate cellular material transport, and maintain epithelial polarity0. Dietary Gln supplementation increased ZO1 gene expression in the intestine of growing Rex rabbits, a result consistent with findings in weaned piglets0. Therefore, Gln may exert its intestinal barrier function by upregulating the expression of the intestinal epithelial cell tight junction protein ZO1, thereby reducing intestinal epithelial permeability.

Effects of Dietary Gln Supplemental Level on Intestinal Immune Barrier of Growing Rex Rabbits The intestine serves as the largest immune organ in animals, bearing the dual responsibilities of tolerating dietary antigens and immune defense. The main effector factor in the intestinal immune response

system is sIgA secreted by plasmablasts. sIgA strongly binds to antigens, preventing adhesion of harmful antigens such as viruses and bacteria to intestinal epithelium, subsequently triggering intestinal humoral and cellular immunity to effectively exclude or eliminate harmful antigens. Similar to findings in piglets0, dietary supplementation with 0.9% Gln significantly increased sIgA content in duodenal and jejunal mucosa but had no significant effect on sIgA content in ileal mucosa of growing Rex rabbits, indicating that the proximal small intestine (duodenum and jejunum) of rabbits is more sensitive to Gln. Additionally, Gln can serve as an energy source and metabolic precursor for immune cells in the intestinal lamina propria, maintaining normal lymphocyte proliferation and differentiation, as well as the ratio of helper T cells to suppressor T cells, as studies have shown that the differentiation of B cells into sIgA-secreting plasma cells is influenced by helper T cells, suppressor T cells, and the cytokines they produce0.

In conclusion, dietary Gln supplemental level did not affect fur quality but improved intestinal mechanical barrier and immune barrier function in growing Rex rabbits. Under the conditions of this experiment, the appropriate dietary Gln supplemental level for weaned to 3-month-old Rex rabbits is 0.9%.

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Note: Figure translations are in progress. See original paper for figures.