Effects of Glutamine on Intestinal Development in Weaned Meat Rabbits (Postprint)

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Abstract

This experiment aimed to investigate the effects of glutamine on intestinal development in meat rabbits. A total of 360 28-day-old weaned New Zealand White rabbits were randomly allocated to 4 groups with 9 replicates per group and 10 rabbits per replicate. The four groups were fed experimental diets supplemented with 0 (control), 0.4%, 0.8%, and 1.2% glutamine on top of the basal diet for a period of 4 weeks. The results showed that dietary glutamine supplementation had no significant effect on stomach weight, cecal weight, and small intestine length at 35, 42, and 56 days of age, nor on small intestine weight at 56 days of age ($P > 0.05$). However, dietary supplementation with 0.8% glutamine significantly increased small intestine weight at 35 and 42 days of age ($P < 0.05$). Dietary supplementation with 0.8% and 1.2% glutamine significantly increased duodenal and jejunal villus height at 35 and 42 days of age ($P < 0.05$), while dietary supplementation with 0.8% glutamine also significantly increased ileal villus height at 35 and 42 days of age, as well as duodenal and jejunal villus height at 56 days of age ($P < 0.05$). Dietary supplementation with 0.8% glutamine significantly decreased duodenal and jejunal crypt depth at 35 and 42 days of age, and ileal crypt depth at 35 days of age ($P < 0.05$). Thus, dietary glutamine supplementation can promote intestinal development in meat rabbits during the first 1–2 weeks post-weaning, and under the conditions of this experiment, the appropriate dietary supplementation level of glutamine for meat rabbits is 0.8%.

Full Text

Effects of Glutamine on Intestinal Development of Weaned Meat Rabbits

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Abstract: This experiment was conducted to evaluate the effects of glutamine on intestinal development in meat rabbits. A total of 360 28-day-old weaned meat rabbits were randomly divided into 4 groups with 9 replicates per group and 10 rabbits per replicate. The four groups were fed experimental diets supplemented with 0 (control), 0.4%, 0.8%, and 1.2% glutamine based on a basal diet for a 4-week period. The results showed that glutamine supplementation had no significant effects on stomach weight, cecum weight, or small intestine length at 35, 42, and 56 days of age, nor on small intestine weight at 56 days of age ($P>0.05$). However, dietary supplementation with 0.8% glutamine significantly increased small intestine weight at 35 and 42 days of age ($P<0.05$). Supplementation with 0.8% and 1.2% glutamine significantly elevated duodenum and jejunum villus height at 35 and 42 days of age ($P<0.05$), while 0.8% glutamine also significantly increased ileum villus height at 35 and 42 days of age and duodenum and jejunum villus height at 56 days of age ($P<0.05$). Dietary supplementation with 0.8% glutamine significantly reduced duodenum and jejunum crypt depth at 35 and 42 days of age and ileum crypt depth at 35 days of age ($P<0.05$). In conclusion, glutamine supplementation can promote intestinal development in meat rabbits during the first 1–2 weeks post-weaning. Under the conditions of this experiment, the suitable dietary glutamine supplementation level for meat rabbits is 0.8%.

Key words: glutamine; meat rabbits; intestinal development

Introduction

In modern intensive meat rabbit production, weaning stress can cause intestinal mucosal damage, decreased intestinal immune function, and reduced digestive enzyme activity, leading to indigestion, diarrhea, growth retardation, and even death[1]. According to statistics, the mortality rate of meat rabbits during growth in China reaches approximately 20%, with over 70% of deaths caused by diarrhea resulting from intestinal mucosal damage and decreased immune function due to weaning stress, representing substantial economic losses[2]. Research has shown that nutritional regulation strategies can effectively alleviate intestinal mucosal damage and immune dysfunction caused by weaning stress[3]. Previously, the primary measure to prevent weaning stress was antibiotic supplementation in diets, but antibiotic residues pose safety concerns for rabbit meat products[4]. In recent years, using nutritional additives to protect the intestinal mucosal barrier and enhance intestinal immune function has become a research hotspot for preventing weaning diarrhea at its source.

Glutamine (Gln) is a conditionally essential amino acid with special nutritional functions that provides energy and nitrogen sources for small intestinal mucosal

cell differentiation, thereby promoting the repair of damaged intestinal mucosa and maintaining normal intestinal immune function. However, weaning interrupts the supply of maternal glutamine to offspring, and their limited capacity to synthesize glutamine often leads to deficiency. Therefore, exogenous glutamine supplementation is crucial for alleviating weaning stress, promoting intestinal mucosal immunity, and improving intestinal immune function in weaned animals[3-10].

Numerous studies have investigated glutamine in piglets and poultry, demonstrating that it can maintain normal intestinal morphology, promote nutrient absorption, enhance mucosal immune function, and improve gut health, thereby effectively mitigating weaning stress[3,9-11]. Currently, research on glutamine application in rabbits is limited and primarily focused on rex rabbits[12-13], with few reports on its effects on meat rabbit intestinal development and optimal dietary supplementation levels. This experiment aimed to investigate the effects of dietary glutamine supplementation on intestinal development in weaned meat rabbits, providing a reference for scientific diet formulation.

Materials and Methods

1.1 Experimental Design

A total of 360 28-day-old weaned New Zealand White rabbits were selected and randomly divided into 4 groups with 9 replicates per group and 10 rabbits per replicate (half male and half female). The four groups were fed experimental diets supplemented with 0 (control), 0.4%, 0.8%, and 1.2% glutamine based on a basal diet for a 4-week experimental period. Dietary glutamine supplementation levels were established based on previous studies from our laboratory[2] and other researchers[12-13], with alanine added to achieve isonitrogenous regulation across groups. At 35, 42, and 56 days of age, 6 rabbits per group (half male and half female) with body weights close to the group mean were selected and slaughtered 1 hour after feeding at 07:00 to collect intestinal samples for developmental index determination.

1.2 Basal Diet and Management

The basal diet was formulated according to nutrient levels recommended by Gu Zilin et al.[1] and local feed resources, and processed into pellets (3 mm diameter, 6–8 mm length). The composition and nutrient levels of the basal diet are presented in Table 1 . Prior to the experiment, rabbit houses were thoroughly cleaned and disinfected. Conventional feeding management and immunization procedures were employed, with free access to feed and water.

1.3 Sample Collection and Measurements

1.3.1 Sample Collection After slaughter by jugular venous exsanguination, the abdominal cavity was immediately opened. The pyloric and ileocecal valves

were ligated, and the digestive tract was removed and carefully separated according to anatomical characteristics. The small intestine was divided into duodenum, jejunum, and ileum based on anatomical features, with each segment ligated separately. A 2-cm middle segment of each intestinal section was excised, rinsed with physiological saline, blotted with filter paper to remove excess water, weighed, and immediately fixed in 10% formalin solution.

1.3.2 Digestive Organ Weight and Small Intestine Length Measurement The small intestinal loops were carefully separated from the mesentery, and their natural length was measured with a soft ruler as the small intestine length. The stomach, small intestine, and cecum were emptied of contents, blotted with filter paper to remove excess water, and weighed to obtain stomach, small intestine, and cecum weights.

1.3.3 Small Intestinal Mucosal Morphology Measurement Paraffin sections were prepared using conventional methods and stained with hematoxylin-eosin. Villus height and crypt depth were measured according to the method of Sun et al.[14]. Villus height was measured from the villus tip to the crypt opening, while crypt depth was measured from the invagination between adjacent villi.

1.4 Statistical Methods

Experimental data were processed using Excel 2007 software and subjected to one-way ANOVA using SPSS 17.0 statistical software. Duncan' s multiple comparison test was applied when significant differences were detected. Significance was set at $P<0.05$, and results are expressed as "mean ± standard deviation" .

Results

2.1 Effects of Glutamine on Digestive Organ Weight and Small Intestine Length

As shown in Table 2 , dietary supplementation with different levels of glutamine had no significant effects on stomach weight, cecum weight, and small intestine length at 35, 42, and 56 days of age, nor on small intestine weight at 56 days of age ($P>0.05$). However, compared with the control group, dietary supplementation with 0.8% glutamine significantly increased small intestine weight at 35 and 42 days of age ($P<0.05$).

2.2 Effects of Glutamine on Small Intestinal Villus Height

As shown in Table 3 , the 0.8% and 1.2% groups exhibited significantly higher duodenum and jejunum villus heights at 35 and 42 days of age compared with the control group ($P<0.05$). The 0.8% group also showed significantly higher duodenum and jejunum villus heights at 56 days of age ($P<0.05$), while no

significant differences were observed among other groups for these parameters (P>0.05). The 0.8% group had significantly higher ileum villus height at 35 and 42 days of age compared with the control group (P<0.05), though no significant differences in ileum villus height were found among groups at 56 days of age (P>0.05).

2.3 Effects of Glutamine on Small Intestinal Crypt Depth

As shown in Table 4 , the 0.8% group exhibited significantly lower duodenum and jejunum crypt depths at 35 and 42 days of age compared with the control group (P<0.05), with no significant differences from the 0.4% and 1.2% groups (P>0.05). No significant differences in duodenum and jejunum crypt depths were observed among groups at 56 days of age (P>0.05). The 0.8% group had significantly lower ileum crypt depth at 35 days of age compared with the control group (P<0.05), without significant differences from the 0.4% and 1.2% groups (P>0.05). No significant differences in ileum crypt depth were found among groups at 42 and 56 days of age (P>0.05).

Discussion

3.1 Effects of Dietary Glutamine Supplementation Level on Digestive Organ Weight and Small Intestine Length

The weights of the stomach, small intestine, and cecum, along with small intestine length, reflect the digestive capacity of meat rabbits, particularly during the first 1–2 weeks post-weaning. Glutamine and glucose serve as primary energy sources for intestinal mucosal cell metabolism and play crucial roles in small intestinal development[15]. In this experiment, dietary supplementation with 0.8% glutamine significantly increased small intestine weight during the first and second weeks post-weaning, but had no significant effect on small intestine weight in the third week. Zhou Lianggao et al.[16] reported that glutamine significantly increased small intestine weight and length in broilers at 2–3 weeks of age, but showed no significant effects at 4 weeks of age, with similar findings reported by Lu Jing et al.[2] in broilers. Soltan[17] noted that glutamine effectively promotes intestinal development in poultry during early growth stages but has minimal effects during later stages. These findings are consistent with our results, indicating that the effects of glutamine on the intestine are developmental stage-dependent, primarily acting during early development. Adult animals can synthesize sufficient glutamine, and suckling rabbits can obtain glutamine from maternal milk. However, weaned rabbits not only lose this maternal source but also experience increased glutamine requirements due to weaning stress, while their capacity to synthesize glutamine remains limited. This may explain why glutamine exhibited significant effects during the first and second weeks post-weaning but had minimal impact during the third week.

3.2 Effects of Glutamine on Small Intestinal Mucosal Villus Height and Crypt Depth

Research has shown that weaning stress can significantly reduce small intestinal mucosal villus height and increase crypt depth in meat rabbits, typically requiring 2–3 weeks to restore normal mucosal structure after early weaning[2]. Studies indicate that glutamine is a non-essential amino acid and the most abundant free amino acid in mammalian plasma and milk[15]. However, under stress conditions (such as weaning, injury, or burns) or pathological states, endogenous glutamine often cannot meet physiological demands, and glutamine depletion may occur, necessitating exogenous supplementation[18]. Research has demonstrated that glutamine, after oxidation, provides energy for mucosal cell proliferation and can be converted into precursors required for cell proliferation[2]. In this experiment, under weaning stress conditions, dietary supplementation with exogenous glutamine increased small intestinal villus height and decreased crypt depth during the first 1–2 weeks post-weaning, alleviating intestinal mucosal damage caused by weaning stress and promoting intestinal development in meat rabbits. These results are consistent with findings in goats by Liu Yujie et al.[19], in rex rabbits by Fu Zhaohui et al.[12], and in piglets by Cabrera et al.[8]. In this study, the optimal effects on increasing villus height and decreasing crypt depth were achieved at a supplementation level of 0.8%, indicating that the most suitable dietary glutamine level for meat rabbits is 0.8%. Fu Zhaohui et al.[12] reported that average daily gain of growing rex rabbits increased initially then decreased with increasing dietary glutamine levels, peaking at 0.9% supplementation, which is similar to our results. Gao Yuqi et al.[13] found that the optimal dietary glutamine level was approximately 1.6% for young rex rabbits during the first month post-weaning, decreasing to 0.8% during the second month. These discrepancies may be related to differences in dietary acid detergent fiber and amino acid levels in the basal diets. Additionally, the lack of significant effects of dietary glutamine on small intestinal mucosal development at 56 days of age in this experiment may be attributed to the rabbits' ability to synthesize sufficient glutamine endogenously by this age.

Conclusion

Dietary glutamine supplementation can promote intestinal development in meat rabbits during the first 1–2 weeks post-weaning. Under the conditions of this experiment, the suitable dietary glutamine supplementation level for meat rabbits is 0.8%.

References

[1] GU Zilin, QIN Yinghe, REN Keliang, et al. *Chinese Rabbit Science* [M]. Beijing: China Agriculture Press, 2013: 268–431.

[2] GUO Zhiqiang, LI Congyan, XIE Xiaohong, et al. Effects of weaning age on intestinal development of meat rabbits [J]. *Chinese Journal of Animal Nutrition*,

2016, 28(1): 102-108.

[3] LI Xue, WANG Xiaocheng, XIONG Xia, et al. Effects of glutamine on intestinal mucosal renewal of weaned piglets and its mechanism [J]. *Chinese Journal of Animal Nutrition*, 2016, 28(12): 3729-3374.

[4] BLAS C, WISEMAN J. *Rabbit Nutrition* [M]. 2nd ed. Beijing: China Agriculture Press, 2015.

[5] WU G Y, KNABE D A. Free and protein-bound amino acids in sow' s colostrum and milk [J]. *The Journal of Nutrition*, 1994, 124(3): 415-424.

[6] WU G Y. Intestinal mucosal amino catabolism [J]. *The Journal of Nutrition*, 1998, 128(8): 1249-1252.

[7] SON J, LYSSIOTIS C A, YING H Q, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway [J]. *Nature*, 2013, 496(7443): 101-105.

[8] WANG B, WU G Y, ZHOU Z G, et al. Glutamine and intestinal barrier function [J]. *Amino Acids*, 2015, 47(10): 2143-2154.

[9] CABRERA R A, USRY J L, ARRELLANO C, et al. Effects of creep feeding and supplemental glutamine or glutamine plus glutamate (aminogut) on pre-and post-weaning growth performance and intestinal health of piglets [J]. *Journal of Animal Science and Biotechnology*, 2013, 4(1): 29.

[10] YE Yaling, WANG Zirui, YOU Jinming, et al. Effects of alanyl-glutamine on the number of immunoglobulin A plasma cells, secretory immunoglobulin A and interleukin contents in small intestinal mucosa of weaned piglets [J]. *Chinese Journal of Animal Nutrition*, 2015, 27(1): 59-66.

[11] LU Jing, LI Wenli, JIANG Jianyang, et al. Effects of glutamine on small intestinal tissue structure and absorption capacity in broilers [J]. *Chinese Journal of Animal Nutrition*, 2012, 24(2): 291-300.

[12] FU Zhaohui, LI Fuchang, LI Bing, et al. Effects of dietary glutamine supplementation on growth performance, nitrogen metabolism and jejunal mucosal enzyme activities in growing rex rabbits [J]. *Chinese Journal of Animal Nutrition*, 2014, 26(2): 397-403.

[13] GAO Yuqi, REN Zhanjun, HU Zhigang, et al. Effects of dietary glutamine supplementation on immune performance and ileal mucin gene expression in young rex rabbits [J]. *Chinese Journal of Veterinary Science*, 2016, 36(4): 655-660.

[14] SUN X, MCELORY A, WEBB K E, Jr, et al. Broiler performance and intestinal alterations when fed drug-free diets [J]. *Poultry Science*, 2005, 84(8): 1294-1302.

[15] BROSNAN J T, BROSNAN M E. Glutamate: a truly functional amino acid [J]. *Amino Acids*, 2013, 45(3): 413-418.

[16] ZHOU Lianggao, ZHANG Shiyuan, LIU Yanfen, et al. Effects of glutamine on growth performance and immune function in broilers [J]. *Chinese Journal of Animal Nutrition*, 2008, 20(3): 305-310.

[17] SOLTAN M A. Influence of dietary glutamine supplementation on growth performance, small intestinal morphology, immune response and some blood parameters of broiler chickens [J]. *International Journal of Poultry Science*, 2009, 8(1): 60-68.

[18] NRC. *Nutrient Requirements of Swine* [S]. 10th ed. Washington, D.C.: National Academy Press, 1998.

[19] LIU Yujie, LIU Junhua, XU Tingting, et al. Effects of glutamine on cecal mucosal morphology and inflammatory cytokine mRNA expression in dairy goats fed high-concentrate diets [J]. *Chinese Journal of Animal Nutrition*, 2014, 26(2): 513-518.

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