Effects of Methionine Deficiency on Small Intestine Development and Goblet Cells in Broiler Chickens (Postprint)

Authors: Liu Xiaodong, Peng Lilan, He Shengmei, Zhang Sen, Shen Hailiang, Wu Bangyuan

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Abstract

This study aimed to investigate the effects of methionine deficiency on small intestinal development and goblet cells in broiler chickens. A total of 120 healthy 1-day-old chicks [body weight (45 ± 5) g] were randomly divided into 2 groups (6 replicates per group, 10 chickens per replicate), and fed a basal diet (control group) and a methionine-deficient diet (experimental group), respectively. The experiment lasted for 42 d, and samples were collected on days 14, 28, and 42. Villus height, crypt depth, and goblet cell number in each segment of the small intestine were determined using light microscopy and histochemical methods. The results showed that compared with the control group, villus height, crypt depth, and the ratio of villus height to crypt depth in each intestinal segment of broiler chickens in the experimental group were significantly or highly significantly decreased ($P < 0.05$ or $P < 0.01$), and the number of goblet cells in the small intestine was also significantly or highly significantly reduced ($P < 0.05$ or $P < 0.01$). The results suggest that methionine deficiency can lead to decreased villus height and crypt depth in the small intestine and reduced goblet cell number, thereby impairing normal small intestinal development and function in broiler chickens.

Full Text

Effects of Methionine Deficiency on Small Intestinal Development and Goblet Cells of Broilers

LIU Xiaodong[1], PENG Lilan[1], HE Shengmei[2], ZHANG Sen[1], SHEN Hailiang[1], WU Bangyuan[2]*

[1]Qiandongnan Vocational & Technical College for Nationalities, Kaili 556000, China

[2]Key Laboratory of Southwest China Wildlife Resources Conservation of Ministry of Education, College of Life Science, China West Normal University, Nanchong 637009, China

Abstract

This study investigated the effects of methionine deficiency on small intestinal development and goblet cells in broiler chickens. One hundred twenty one-day-old healthy chicks with a body weight of (45$±$5) g were randomly allocated into two groups (six replicates per group, ten birds per replicate). Birds in the control group were fed a basal diet, while those in the experimental group received a methionine-deficient diet for 42 days. Samples were collected on days 14, 28, and 42 to measure villus height, crypt depth, and goblet cell counts in different small intestinal segments using light microscopy and histochemical methods. The results demonstrated that compared with the control group, the experimental group exhibited significantly or extremely significantly decreased villus height, crypt depth, and villus height-to-crypt depth ratio ($P<0.05$ or $P<0.01$) across all intestinal segments, along with significantly or extremely significantly reduced goblet cell numbers ($P<0.05$ or $P<0.01$). These findings indicate that methionine deficiency impairs normal intestinal development and compromises functional integrity in broiler chickens by reducing villus height, crypt depth, and goblet cell populations.

Keywords: methionine deficiency; small intestinal development; villus height; crypt depth; goblet cell; broilers

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Introduction

Methionine is closely associated with the metabolism of various sulfur-containing compounds in living organisms and participates in the synthesis of new proteins. Rapid animal growth is directly proportional to the rate of protein synthesis, which forms the essential foundation for intestinal growth and development. Amino acids promote intestinal development—for instance, 4.82% branched-chain amino acids can enhance intestinal growth in broilers, and exogenous glutamine supplementation significantly promotes early intestinal development in broiler chicks. Additionally, dietary crude protein levels influence swine performance and gastrointestinal development. Therefore, methionine likely plays a crucial role in animal intestinal growth and development.

Methionine requirements vary among different species, ages, physiological states (such as gestation, lactation, and early growth), and sexes. Inadequate methionine supply impairs animal growth performance and physiological functions. Methionine deficiency reduces body weight, feed intake, and feed conversion efficiency in broilers, decreases protein synthesis, and affects cellular oxidative function. It also damages immune organs and alters enzyme activities in vital

organs such as the heart and liver, causing severe hepatic tissue injury. However, research on the effects of methionine deficiency on intestinal development and function remains limited.

The intestine serves as the primary interface between the internal and external environments, performing critical functions. The small intestinal mucosa provides an immune barrier that prevents invasion by intestinal microbes and toxins, while also serving as a major organ for digestion and absorption of nutrients, electrolytes, and water, and secreting enzymes and transport proteins. The luminal mucosal system constitutes an important component of the immune system, playing a vital role in host defense. Previous studies have established that intestinal absorptive function is closely correlated with morphology, and any morphological alterations can impair intestinal function. Therefore, this study employed light microscopy to examine small intestinal villus height and crypt depth, along with histochemical methods to quantify goblet cell numbers, thereby investigating the impact of methionine deficiency on small intestinal development and function in broilers. These findings may provide valuable reference for understanding methionine' s effects on intestinal function in humans and other animals.

Materials and Methods

1.1 Experimental Diets and Animals One hundred twenty one-day-old healthy Cobb broiler chicks with a body weight of (45$±$5) g were purchased from Chengdu Wenjiang Zhengda Livestock and Poultry Co., Ltd. in Sichuan Province. A corn-soybean meal basal diet served as the control diet, while the experimental diet consisted of the basal diet without supplemental methionine. Diets were formulated according to NRC broiler nutrition standards, with composition and nutrient levels presented in Table 1 .

1.2 Reagents and Instruments **Reagents:** 4% paraformaldehyde, graded ethanol series (50%, 65%, 70%, 75%, 85%, 90%, 95%, and 100%), hematoxylin, eosin, periodic acid, and Schiff' s reagent.

Instruments: Paraffin microtome (LEICA KD-2258, Jinhua Kedi Instrument Equipment Co., Ltd., China), light microscope (OLMPUS BA410, Motic Group Co., Ltd., China), microscopic imaging system (MoticamPro205A, Nikon, Japan), and Image Pro Plus v6.0 software.

1.3 Experimental Procedures

1.3.1 Animal Grouping and Management The 120 broiler chicks were randomly divided into two groups with six replicates per group and ten birds per replicate. One replicate from each group was designated for continuous observation throughout the trial. During the experimental period, the two groups were fed the basal diet and methionine-deficient diet, respectively. Birds were

housed in stainless steel experimental cages, with one cage per replicate, and provided ad libitum access to water and feed. Management practices followed conventional brooding protocols. The trial lasted 42 days, with sampling conducted on days 14, 28, and 42.

1.3.2 Histological Observation On days 14, 28, and 42, five birds from each group were randomly selected and euthanized. Approximately 1 cm tissue samples were collected from the middle segments of the duodenum, jejunum, and ileum, fixed in 4% paraformaldehyde, rinsed under running water, and dehydrated through graded ethanol series (75%, 85%, 95%, and 100%). Samples were then cleared with xylene, infiltrated with paraffin, and embedded. Five-micrometer thick sections were prepared, mounted, and stained with hematoxylin-eosin (HE). Villus height and crypt depth were measured under a light microscope from ten typical fields (with intact, straight villi) per sample, and the average values were calculated to determine the villus height-to-crypt depth (V/C) ratio.

1.3.3 Histochemical Observation of Intestinal Goblet Cells Sample collection and section preparation followed the same protocol as histological observation. The periodic acid-Schiff-Alcian blue staining method was employed. The staining procedure included: conventional dewaxing of paraffin sections to water; Alcian blue staining for 5 minutes (dropwise); rinsing with tap water followed by distilled water; periodic acid treatment for 2-5 minutes, then washing; Schiff' s reagent staining for 30 minutes (or longer), followed by 10 minutes of washing under gentle running water; optional counterstaining with alum hematoxylin for approximately 1 minute, differentiation, and bluing; final washing, dehydration, clearing, and mounting. Goblet cells stained blue (acidic mucin), magenta (neutral mucin), or blue-magenta (mixed acidic-neutral mucin).

Microscopic photography and Image Pro Plus software were used for goblet cell counting. Specifically, five periodic acid-Schiff-Alcian blue stained paraffin sections were prepared from each intestinal segment per bird, with five random fields examined per section. Goblet cell numbers were counted, and data were analyzed statistically using Excel.

1.4 Statistical Analysis Experimental data were analyzed using SPSS 16.0 software. All values were expressed as mean ± standard deviation. Independent samples t-test was used to compare differences between the experimental and control groups. $P<0.05$ was considered statistically significant, and $P<0.01$ was considered extremely significant.

Results

2.1 Effects of Methionine Deficiency on Small Intestinal Morphology

2.1.1 Effects of Methionine Deficiency on Duodenal Morphology As shown in Table 2 , villus height in the duodenum was significantly or extremely significantly lower in the experimental group compared with the control group on days 28 and 42 ($P<0.05$ or $P<0.01$). Crypt depth was significantly reduced in the experimental group on days 14, 28, and 42 ($P<0.05$). The V/C ratio was also significantly decreased in the experimental group on day 42 ($P<0.05$).

2.1.2 Effects of Methionine Deficiency on Jejunal Morphology Methionine deficiency affected jejunal morphology similarly to the duodenum. As shown in Table 3 , villus height was significantly or extremely significantly lower in the experimental group compared with the control group on days 14, 28, and 42 ($P<0.05$ or $P<0.01$). Crypt depth was extremely significantly reduced in the experimental group on day 42 ($P<0.01$). The V/C ratio was significantly decreased in the experimental group on days 28 and 42 ($P<0.05$).

2.1.3 Effects of Methionine Deficiency on Ileal Morphology As shown in Table 4 , ileal villus height was extremely significantly reduced in the experimental group compared with the control group on days 14, 28, and 42 ($P<0.01$). Crypt depth was significantly or extremely significantly lower in the experimental group ($P<0.05$ or $P<0.01$). The V/C ratio was significantly or extremely significantly decreased in the experimental group on days 14 and 42 ($P<0.05$ or $P<0.01$).

2.2 Effects of Methionine Deficiency on Small Intestinal Goblet Cell Numbers Histochemical analysis revealed that goblet cells were primarily distributed among intestinal villus epithelial cells (Figure 1 [Figure 1: see original paper]–Figure 3 [Figure 3: see original paper]) and within crypts (Figure 4 [Figure 4: see original paper]–Figure 6 [Figure 6: see original paper]).

As shown in Figure 1, goblet cell numbers among duodenal villus epithelial cells were significantly or extremely significantly reduced in the experimental group compared with the control group on days 28 and 42 ($P<0.05$ or $P<0.01$). As shown in Figure 2 [Figure 2: see original paper], goblet cell numbers among jejunal villus epithelial cells were extremely significantly lower in the experimental group on day 42 ($P<0.01$). As shown in Figure 3, goblet cell numbers among ileal villus epithelial cells were significantly reduced in the experimental group on day 42 ($P<0.05$).

As shown in Figure 4, goblet cell numbers within duodenal crypts were extremely significantly reduced in the experimental group on day 42 ($P<0.01$). As shown in Figure 5 [Figure 5: see original paper], goblet cell numbers within jejunal crypts were significantly or extremely significantly lower in the experimental group on days 28 and 42 ($P<0.05$ or $P<0.01$). As shown in Figure 6, goblet cell numbers within ileal crypts were also extremely significantly reduced in the experimental group on day 42 ($P<0.01$).

Discussion

The intestine serves as a critical interface between the internal and external environments, with the small intestinal mucosa providing essential immune barrier functions that prevent microbial and toxin invasion. As a vital digestive and absorptive organ, the small intestine facilitates nutrient, mineral salt, and water absorption while secreting enzymes and transport proteins. The integrity of intestinal villi determines digestive and absorptive capacity and protects the organism from harmful substances. Previous research has demonstrated that dietary methionine supplementation helps maintain the barrier function of small intestinal mucosa in weaned piglets. Our findings indicate that methionine deficiency impairs intestinal development and barrier function, manifested by reduced villus height, crypt depth, V/C ratio, and decreased goblet cell populations.

Villus height, crypt depth, and V/C ratio are direct indicators of the intestinal environment and important parameters reflecting gut health. The V/C ratio has been validated as a reliable indicator for evaluating intestinal developmental status. This study revealed that dietary methionine deficiency negatively affected villus cells in the duodenum, jejunum, and ileum, causing shortened villus height, reduced crypt depth, and decreased V/C values. Increased villus height correlates with enhanced intestinal digestive and absorptive function by expanding the surface area for absorption, increasing brush border enzyme expression and activity, and improving nutrient transport capacity. We hypothesize that methionine deficiency may impair brush border enzyme expression and nutrient metabolism, ultimately diminishing intestinal nutrient transport and absorptive capacity. These results demonstrate that methionine deficiency damages small intestinal structure, impedes growth and development, inhibits nutrient absorption, and consequently reduces animal performance and normal growth. Additionally, since the intestinal mucosal immune system contains various immune cells involved in immune mechanisms, methionine deficiency may also compromise immune function.

Goblet cells are widely distributed among intestinal epithelial cells and secrete mucus, synthesizing and releasing large molecular weight glycoconjugates known as mucins. Mucins are glycoproteins that form a lubricating mucus layer on the epithelial surface, providing protective functions as the first barrier for intestinal integrity and health. Our results showed that goblet cell numbers in the duodenum, jejunum, and ileum were significantly lower in the methionine-deficient group compared with the control group. Goblet cell populations vary with intestinal segment, dietary habits, management practices, pathogen invasion, developmental stage, and species. By comparing the methionine-deficient and control groups, we speculate that reduced goblet cell numbers may also relate to protein deficiency (as methionine is the initiating amino acid for protein synthesis). Previous studies have reported that increasing plant protein levels in rainbow trout diets increased intestinal goblet cell numbers. Our study demonstrates that methionine deficiency reduces goblet cell populations in broiler small

intestine, negatively affecting mucin secretion and ultimately altering the structural composition and physicochemical properties of the mucus layer. This indicates compromised protective function of the intestinal mucus layer and impaired barrier function. Damage to the intestinal barrier mediated by mucin can affect tight junction permeability and immune regulation, potentially promoting the development of inflammatory, ulcerative, and neoplastic intestinal diseases.

Conclusion

Methionine deficiency decreases villus height, crypt depth, and V/C ratio in the small intestine (duodenum, jejunum, and ileum) of broilers and reduces goblet cell numbers. These findings indicate that methionine deficiency impedes normal small intestinal development and compromises its functional integrity.

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