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Effect of supplementation of betaine hydrochloride on gut health in broilers

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Abstract

An experiment was carried out using 120 day-old Cobb broiler chicks to evaluate the impact of betaine hydrochloride supplementation on gut health. The chicks were randomly allotted to four dietary treatments (T₁ to T₄), each consisting of three replicates with ten birds, and reared for 42 days. The control group (T₁) received a basal diet formulated as per BIS (2007) ^[2] standards, whereas T₂, T₃, and T₄ diets were supplemented with 0.1%, 0.2% and 0.25% betaine hydrochloride, respectively. The results indicated that dietary inclusion of betaine hydrochloride significantly enhanced villus height and crypt depth in the duodenum, jejunum, and ileocaecocolic junction. Additionally, it led to a reduction in *Escherichia coli* counts and an increase in *Lactobacillus* populations compared with the control group at the end of the trial. It was therefore concluded that betaine hydrochloride supplementation positively influences gut morphology and microbial balance in broiler chickens.

Keywords: Betaine hydrochloride, *Escherichia coli*, *Lactobacillus*, gut microbial load, gut morphology and gut health

Introduction

India's poultry industry has witnessed rapid expansion, playing a crucial role in the nation's agricultural development. The country now ranks fifth globally in meat production, with output increasing from 6.69 million tonnes in 2014-15 to 10.25 million tonnes in 2023-24, representing a compound annual growth rate (CAGR) of 4.85%. Livestock continues to be a cornerstone of rural livelihoods, providing both income and employment opportunities to marginalized communities. Poultry meat, consisting mainly of water and fat, serves as an excellent source of high-quality protein, iron, selenium, zinc, and B-complex vitamins. (Chand *et al.*, 2017) ^[3].

India's poultry sector, largely driven by commercial enterprises, is undergoing rapid expansion due to the increasing demand for affordable protein sources. To improve production efficiency, various feed additives are employed, with betaine gaining prominence as an important supplement. Betaine, a trimethyl derivative of glycine naturally present in plant and animal tissues, possesses both carboxylate and quaternary ammonium functional groups (Shakeri *et al.*, 2020) ^[10]. It serves as a methyl donor in the conversion of homocysteine to methionine and acts as a non-ionic osmolyte that helps maintain cellular hydration (Day, 2016) ^[4]. Typically derived from sugar beet molasses, betaine has demonstrated multiple benefits in broilers, including enhanced growth performance, improved gut health, better coccidiosis management, alleviation of heat stress, and strengthened immune response (Ghasemi and Nari, 2020) ^[6].

At the metabolic level, betaine serves as a methyl group donor in transmethylation reactions that are essential for the synthesis of compounds such as creatine and carnitine, thereby potentially lowering the dietary requirements for methionine and choline (Eklund *et al.*, 2005) ^[5]. Supplementation with betaine hydrochloride has been shown to increase villus height and the villus height-to-crypt depth ratio, thereby improving nutrient absorption and expanding the intestinal surface area (Hassan *et al.*, 2005) ^[7].

When included in feed or water, betaine helps maintain hydration and energy balance (Eklund *et al.*, 2005) [5]. Furthermore, its osmoprotective properties contribute to enhanced intestinal development and better nutrient utilization (Afrin *et al.*, 2018) [1].

Accordingly, this study was undertaken to evaluate the impact of dietary betaine hydrochloride on broiler gut health.

Materials and Methods

The present study was conducted at the Department of Poultry Science, Veterinary College, Hebbal, Bengaluru. A total of 120 day-old commercial broiler chicks were procured from Venkateshwara Hatcheries Pvt. Ltd., and betaine hydrochloride was obtained from Higain Feeds & Farms Pvt. Ltd., Mandya. Upon arrival, the chicks were individually weighed and randomly allocated into four dietary treatment groups, each consisting of three replicates with ten chicks per replicate. Following the Bureau of Indian Standards (BIS, 2007) [2] guidelines, the control group (T₁) was fed a basal diet, while T₂, T₃, and T₄ were supplemented with 0.1%, 0.2%, and 0.25% betaine hydrochloride, respectively, for a duration of 42 days.

The trial lasted six weeks, during which the birds were reared under a deep litter system with ad libitum access to feed and water. Standard management practices were maintained to ensure optimal bird health and welfare. Vaccinations against Marek's disease (HVT strain), Newcastle disease (Live BI strain), and Infectious Bursal Disease (intermediate strain) were administered using vaccines supplied by Ventri Biologicals, Bengaluru. Ethical approval for the experiment was obtained from the Institutional Animal Ethics Committee of KVAFSU, Bidar, Karnataka. The study aimed to assess the effect of different dietary inclusion levels of betaine hydrochloride on gut microbial load and intestinal morphology in broiler chickens.

At the conclusion of the trial, two birds from each replicate of all treatment groups (T₁ to T₄) were sacrificed. Intestinal contents from the small intestine were collected aseptically in sterile containers for microbial enumeration using the standard plate count method (Postgate, 1969) [9]. *E. coli* counts were determined on MacConkey agar, while *Lactobacillus* counts were assessed using Brain Heart Infusion agar by the pour plate technique (Mackie and McCartney, 1996) [8]. Tenfold serial dilutions of intestinal samples were prepared, and bacterial counts were expressed as log colony-forming units (CFU) per gram of intestinal content.

Additionally, tissue samples from the duodenum, jejunum, and ileocecal junction were collected on day 42 from the slaughtered birds. These samples were flushed with buffered saline and fixed in 10% neutral buffered formalin for histopathological evaluation, focusing on parameters such as villus height and crypt depth.

Results

The results of the present study indicated a significant ($p < 0.05$) influence of dietary betaine hydrochloride supplementation on gut microbial load and intestinal morphology in broiler chickens. The villus height (μm) of the duodenum in treatment groups T₁, T₂, T₃, and T₄ was recorded as 1169.50, 1221.17, 1230.50, and 1232.17, respectively, while the corresponding crypt depths (μm) were 142.00, 191.33, 196.83, and 196.83 at the end of the 42-day feeding trial. In the jejunum, villus heights (μm) were 910.33, 961.33, 958.33, and 958.33 for T₁, T₂, T₃, and T₄, respectively, with

crypt depths (μm) of 125.00, 181.33, 175.00, and 174.83. Similarly, in the ileocaecocolic junction, the villus heights (μm) were 654.17, 714.33, 711.17, and 703.00, while the crypt depths (μm) measured 124.50, 172.33, 179.17, and 171.17 for groups T₁, T₂, T₃, and T₄, respectively.

At the conclusion of the experiment, the intestinal *E. coli* count ($\log_{10}$ CFU/g) for T₁, T₂, T₃, and T₄ was 7.64, 6.71, 6.78, and 6.74, respectively, indicating a reduction in *E. coli* population with betaine supplementation. Conversely, the *Lactobacillus* count ($\log_{10}$ CFU/g) increased to 7.00, 7.02, and 7.01 in T₂, T₃, and T₄, compared with 6.66 in the control group (T₁). These findings suggest that betaine hydrochloride supplementation positively influenced gut morphology and microbial balance in broilers.

Discussion

The results of the present study revealed significant difference ($p < 0.05$) in gut morphology and gut microbial load of birds in the groups fed with 0.1, 0.2 and 0.25% betaine hydrochloride in basal diet in comparison to the control group.

The current findings were in agreement with Song *et al.* (2021) [12], who concluded that supplementation of betaine hydrochloride (1000 mg per kg) in broiler diet showed significant increase ($p < 0.05$) in the villus height of jejunum and ileum compared to the control group. They reported the positive impact may be attributed to betaine enhancing intestinal villus growth by dose-dependently upregulating IGF-1 and EGF, stimulating enterocyte proliferation and strengthening tight junctions through increased occludin and claudin-1 expression which in turn improves the gut morphology. The study was also in accordance with Shubhnish *et al.* (2025) [11], who reported that gut morphology had significantly ($p < 0.05$) improved by betaine supplementation (2.0 g per kg), with increased villi length and villi height: Crypt depth ratio compared to the control group. They noted the positive impact may be due to betaine's ability in preserving enterocyte integrity during heat stress by maintaining cellular hydration, preventing villi atrophy and crypt hyperplasia caused by summer stress.

The current findings were in agreement with Sun *et al.* (2019) [13], who reported that betaine supplementation (0.5 g per kg) significantly increased the microbial population of *Lactobacillus* and *Bifidobacterium* ($p < 0.05$) and decreased *Clostridium* population ($p < 0.05$) compared to the control group. They noted the findings may be due to betaine (500 mg/kg) serving as a methyl donor for *Lactobacillus* and *Bifidobacterium* metabolism, upregulating their folate biosynthesis pathway, while inhibiting *Clostridium perfringens* growth by disrupting glycine betaine transport systems.

Conclusion

Based on the above results, it was concluded that the addition of 0.1, 0.2 and 0.25% betaine hydrochloride in basal diet was beneficial in improving the gut health in broilers. However, since there was no significant difference among 0.1, 0.2 and 0.25% betaine hydrochloride on gut health, it was concluded that addition of 0.1% betaine hydrochloride is beneficial in improving gut health in broilers.

Conflict of Interest

Not available

Financial Support

Not available

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