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Research Article

The Effect of the Water Additive KimchiStock® as an Herbal Growth Promoter on the Jejunal Histological and Ultrastructural Changes of Broiler Chickens

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Abstract

Background and Objective: There has been a great deal of attention on the use of herbal feed additives for replacing the utility of antimicrobial agents. The objective of the present study was to investigate the effect of adding KimchiStock® to drinking water on the histological and ultrastructural changes of the villi in the jejunum of broiler chickens. **Materials and Methods:** Fifty one-day-old broiler chickens, strain Cobb, were used as experimental animals. Chickens were randomly allotted into control (K1) and treated (K2) groups. K2 received a 0.2% water additive (KimchiStock®) in their drinking water for 5 days consecutively every week for 5 weeks. At the end of the treatment periods, the chicks in each group were killed by cervical dislocation and necropsied and jejunal samples were collected for histological and ultrastructural analyses. **Results:** Based on the description of the jejunal histopathology, the best results were found in the treated group. In the ultrastructural study, although there were no significant differences ($p>0.05$) between treatments for villi width; the height and the density of the jejunal villi in the treated (K2) group were significantly ($p<0.05$) higher and more dense compared to those in the control (K1) group. **Conclusion:** KimchiStock® increased immunomodulation through hyperplasia of lymphocytic cells, which allows it to prevent infection in poultry. KimchiStock® increases the height and density of the jejunal villi which helps maximize digestion capacity and growth performance. Thus, KimchiStock® can be used as a substitute for growth promoter antibiotics.

Key words: Broiler, herbal growth promoter, jejunal villi, ultrastructure, water additive

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

To increase production of commercial broilers, the use of antibiotics as growth promoters has become an important strategy used by the poultry producers. The increased risk of resistance of pathogens to antibiotics and the accumulation of antibiotic residues in poultry products (meat, eggs)[1,2] have made some countries to start banning the use of sub-therapeutic doses of antibiotic(s) as additives in the chicken feed[3]. On the other hand, consumers awareness about this issue has led scientists and nutritionists want to focus on finding alternatives to antibiotic growth promoter (AGP) that guarantee the safety of poultry products[4]. Several studies have studied the potency of natural growth promoters (NGPs) such as probiotics, prebiotics, synbiotics, exogenous enzymes, organic acids and phytogenic feed additive (PFD) that can improve the intestinal health and productivity[5]of poultry. Some NGPs that have been used in broilers are biotechnology products of herbs or functional foods in which the active compound has been used in humans.

Kimchi is a traditional Korean foods that has many health benefits for humans. The functional benefits of kimchi for human health have been proven *in vitro* and *in vivo* using rats and mice as experimental animals[6]. The fact that the number of research studies on the potency of kimchi for human health has significantly increased has led to the consideration of using kimchi as an alternative to antibiotic growth promoters in livestock. The Nutritional and health values of kimchi may be due to its low calories content (18 kcal/100 g) as well as its content of vitamins, minerals and fiber which are important for the body. In addition, kimchi is also a good source of phytochemicals including β-cytosterol compounds, glucosinolates, isothiocyanates, indoles, allyl compounds etc. and contains some lactic acid bacteria that act as probiotics such as *Lactobacillus plantarum, Lactobacillus brevis* and *Leuconostoc mesenteroides*[7,8]. According to Award *et al.*[9] lactobacilli and bifidobacteria are believed to have some effects on GI tract histology and ultrastructure. In another study, Thanh *et al.*[10] proved that adding several combinations of *Lactobacillus* metabolite to the diet of broilers significantly increased the height of the villi of the duodenum, jejunal and ileum compared to those in the control group. In addition, Thu *et al.*[11] showed that a combination of *Lactobacillus* metabolites as a water additive for pigs significantly increased the height of duodenal microvilli and the height of jejunal microvilli in the treated group when provided in drinking water. The jejunal is the part of the small intestine that is responsible for absorbing nutrients (carbohydrates, proteins, lipid) from digested food into the blood stream.

Based on several research results it has been suggested that functional effects of kimchi is come from its raw material components, fermentation products and metabolite compounds produced by lactobacillus bacteria in kimchi culture. Previously, a wide range of phytogenic feed additives were proven to exert the beneficial effect on the digestive tract by increasing digestive enzymes production such as trypsin, amylase and intestinal mucus production[12]. Whether the water additive KimchiStock® also has a positive influence on the digestive tract, especially the jejunal, needs to be studied.

The objective of the present study was to investigate the effect of adding KimchiStock® to drinking water on histological and ultrastructural changes (height, width and density) of the villi in the jejunal of broiler chickens.

MATERIALS AND METHODS

Experimental design: Fifty one-day-old broiler chickens, strain Cobb, that had been given hatchery vaccinations against Newcastle disease (ND), infectious bronchitis-Newcastle disease (IB-ND) and infectious bursal disease (IBD) were used as experimental animals. The chickens were then randomly allotted into 2 groups, the control group (K1) and treated group (K2). K2 received a 0.2% water additive (KimchiStock®) through drinking water for 5 days consecutively every week for 5 weeks. At the age of 1-14 days, all of the chickens were fed a pre-starter B10 ration 4 times per day followed by giving a starter GM-1 MJL ration 3 times per day at the age of 15-35 days. The quantity of the ration given to the animals was adjusted according to the age of the chicken. At the end of the treatment periods, the chicks in each group were killed by cervical dislocation and necropsied and jejunal samples were collected for histological and ultrastructural analyses (Ethical Clearance No. 00083/04/LPPT/VII/2017).

Statistical analysis: Data were subjected to a one-way analysis of variance[13] using SPSS (SPSS for Windows version 22.0).

Histological analysis: For histological studies, 4 cm of the jejunal was collected and fixed in a 10% neutral buffered formalin solution for 24 h. The trimmed cross-sections of the jejunal samples were placed in cassettes and processed on a tissue processor overnight. The tissues were then embedded in paraffin and finally the samples were cut to a thickness of 5 microns using a rotary microtome (Yamato RV-240) and placed on slides. The tissues sections were then stained with hematoxylin and eosin and observed under a light microscope.

Intestinal ultrastructural analysis: During necropsy, approximately 4-cm long pieces of the jejunum were taken out and fixed in a fixative solution (10% neutral buffered formalin). After fixation, the jejunum tissues were processed using a biological sample preparation method[14]. The first step was the cleaning process which was performed by soaking the tissue into a cacodylate buffer for approximately 2 h followed by an agitation process in an ultrasonic cleaner for 10 min. In the next step, the samples were pre-fixed in a 2.5% glutaraldehyde solution for 24 h and this step was followed by another next fixation process in a 2% tannic acids solution for 6 h. After the last fixation, the tissues were washed using a cacodylate buffer four times for 15 min and subsequently washed with aquadest for 15 min.

The next step was dehydration of the tissue samples which was performed by soaking the tissues in a graded concentrations of ethanol solutions (50, 70, 85, 95 and 100%, twenty minutes each). After dehydration, the sample were dried in tert-butanol solution, frozen in a freezer and freeze-dried until they were dry. The samples were placed onto a specimen stub coated with gold (Au) using an ion's coater and examined with a Hitachi SU-3500 scanning electron microscope. The parameters studied were villus height, villus width and villus density.

RESULTS

Histological study: The result of treating K1 (as the control group) and K2 (as the treated group) regarding histopathological changes of the jejunum after 35 days are shown in Fig. 1 and 2. From the description of the jejunal histopathology, the best result was found in the treated group. The histopathological changes observed in the lamina propria of the jejunal mucosa from the treated group that received supplementation with a 0.2% water additive (KimchiStock®) represented hyperplasia of lymphocytic cells. The lymphocytic cells present in the lamina propria intestine are the constituent components of the gut associated lymphoid tissue (GALT). GALT hyperplasia of the treated group (Fig. 2) was observed to be more extensive (widespread) compared to the GALT observed in the control group (Fig. 1).

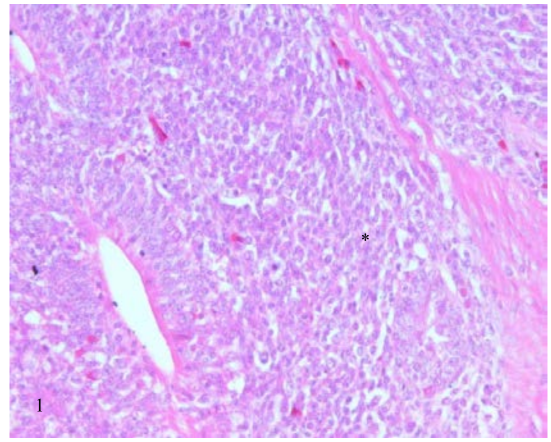

Fig. 1: Histological structure of jejunal gut-associated lymphoid tissue (GALT) from 5-weeks-old broiler chickens in the control group (K1)
No hyperplasia of lymphocytic cells is observed within GALT (*) (Haematoxylin and eosin, 1000×)

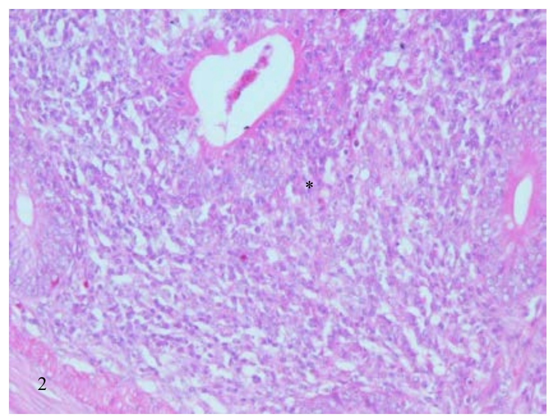

Fig. 2: Histopathological structure of jejunal gut-associated lymphoid tissue (GALT) from 5-weeks-old broiler chickens in the treated group (K2)
Moderate to severe hyperplasia of lymphocytic cells is observed within GALT (*) (Haematoxylin and eosin, 1000×)

Ultrastructural study: Scanning electron microscopy micrographs of the jejunal villi in the present study are presented in Fig. 3 and 4. The effects of adding a 0.2% water additive (KimchiStock®) on the parameters studied are presented in Table 1. Statistically, the height and the density of the jejunal villi in the treated (K2) group were significantly ($p<0.05$) higher compared to the height and the density of the jejunal villi in the control (K1) group. There were no significant differences ($p>0.05$) between treatments for villi width (Table 1).

Table 1: Parameters (height, width and density) of jejunum tissue in broiler chickens at 5 weeks of age

Variable studied	Treatment groups K1 (control)	K2 (0.2% water additive)	Significance
Villus height (µm)	836.50±195.08	1152.00±94.18	0.04 ($p \leq 0.05$)
Villus width (µm)	103.00±26.71	142.40±32.17	0.08 ($p \geq 0.05$)
Villus density (number of villi/area/chicken in 4 electron micrographs segments)	32.75±3.77	65.60±22.14	0.03 ($p \leq 0.05$)

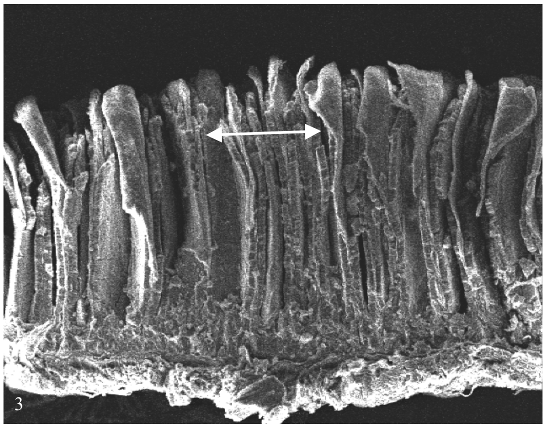

Fig. 3: Scanning electron micrograph of the jejunal villi from a 5-weeks-old broiler chicken in the control group (K1)
The jejunal villi look small, shrunken and not dense (↔) (JSM-5000, MAG 50×, ACCV 20kV)

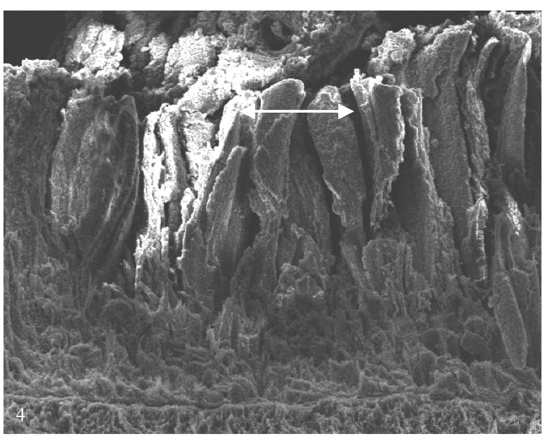

Fig. 4: Scanning electron micrograph of the jejunal villi from a 5-weeks-old broiler chicken in the treated group (K2)
The jejunal villi are thicker, taller and denser than the jejunal villi shown in Fig. 3 (↔) (JSM-5000, MAG 50×, ACCV 20 kV)

DISCUSSION

Kimchi, a traditional fermented Korean food has been reported to have an important role in improving health[15]. Herbal probiotic lactic acid bacteria (LAB) are considered to play an important role in keeping and maintaining health. Many bacteria can play a role in Kimchi fermentation but LAB are more important than other probiotic bacteria because putrefactive bacteria (decay) are unable to reproduce during the herbal fermentation process. In fact, there are additional ingredients in the kimchi, resulting in more useful kimchi, that is able to also kill pathogenic bacteria and even improve kimchi's function[16].

Hyperplasia of lymphocytic cells was significant upon histopathological examination of the jejunum in the lymphoid tissue section or the Gut Associated Lymphoid Tissue (GALT) of the gastrointestinal tract, accompanied by the addition of high-shaped leaves with a broad base of villi and increased villi density in the digestive tract, especially in the jejunum according to scanning electron microscopy in the present study. These histological and ultrastructural findings prove that kimchi has a positive effect on the digestive tract. The results of a similar study were also reported by Chichlowski *et al.*[17] who showed that there was a high likelihood that taller in villi would increase absorption in part of the digestive tract. Based on the results of these studies it was also reported that microbial administration (directly-fed microbes) increased the jejunal height and the jejunal cryptic depth. Increasing the size and number of villi causes the digestive tract's absorptive surface and the nutrient transport systemto be increased[18,19] so that probiotics have a beneficial effect on the host, especially on increasing the performance of body weight, growth and feed efficiency in broilers[20]. In addition, maximum digestion capacity is achieved due to the wide digestive tract lumen with tall villi and adult enterocytes (cells) and this situation is necessary for optimal growth in poultry. The proliferation of intestinal epithelial cells was stimulated by the short chain fatty acid such as propionic acid and butyric acid produced by probiotic lactic acid bacteria[21].

Based on the results of the present study, it is observed that broilers supplemented with a 2% water additive (KimchiStock®) act as immunomodulators characterized by hyperplasia of lymphocytic cells in GALT. Thus, administration of kimchi will prevent the possibility of infections that can be caused by pathogenic microorganisms without interfering or decreasing the performance of chicken growth and thus can act as a substitute for the growth promoter antibiotics. Similar results were reported by Sato *et al.*[22] who found that lactobacillus probiotics, especially *L. gasseri* TL2919 increased the development of immune system T cells via toll-like receptor (TLR) signals which increased the growth and development of the digestive tract immune system[22]. Kimchi has long been known as antibacterial. Many LAB strains that have been isolated from kimchi produce antimicrobial compounds, including: bacteriocin and nicin[23,24]. The LAB species contained in kimchi have strong antimicrobial activity against *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*[25]. The LAB microbes contained in kimchi also act as flavoring and provide the texture and nutrition of broiler products that are needed, in addition to producing bacteriocin as a strong anti microbial agent[26]. The results of recent studies indicated that kimchi is effective in healing natural infections of the avian influenza virus in broilers[27,28]. The results of the present study, which is the first reported on the relevance of kimchi implementation with histological immunomodulation and gastrointestinal ultrastructures in broiler chickens proves that kimchi can improve the health and performance of broiler chickens and act as a substitute for growth promoter antibiotic (AGP).

CONCLUSION

KimchiStock® increases immunomodulation through hyperplasia of lymphocytic cells and prevents infection in poultry. KimchiStock® also increases the height and density of the jejunal villi of the digestive tract allowing optimal absorption of nutrients from digested food. Thus, it is highly possible to use the water additive KimchiStock® as a substitute for growth promoter antibiotics.

SIGNIFICANCE STATEMENT

This study discovered the positive effect of the water additive KimchiStock® on the intestinal health of broilers and showed that KimchiStock® may have a good impact on the growth of broilers.

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