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Research Article
Improvement of Small Intestine Morphometry in Broiler Chicken Using Fermented Cow and Soymilk as Probiotic

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Abstract

Background and Objective: Probiotics have been reported to have positive effect on gut morphology and subsequent performance of poultry birds. Therefore, the present study was carried out to evaluate the effect of fermented soy and cow milk as probiotic on small intestine morphometry of broiler chicken. **Materials and Methods:** 100 day old commercial broiler chicks were allocated to 4 treatment groups in a complete randomized design (CRD) and each treatment was replicated 4 times with 5 chicks in each replicate. The treatments consisted of birds fed: Only basal feed (T0), basal feed with 100% fermented cow milk (T1), basal feed with fermented cow milk+fermented soy milk in 50:50% ratio (T2) and basal feed with fermented cow milk+fermented soy milk with a 75:25% ratio (T3). **Results:** Use of fermented cow or soy milk as probiotic in the diet of broiler had no significant ($p>0.05$) effect on jejunal villus height when compared with the control group. However, there was an improvement in the jujunal villus width and number of villi in chicken fed fermented cow or soy milk as probiotic in the diet when compared with the control. **Conclusion:** Inclusion of dietary fermented cow or soy milk in the diet resulted in improvement in the morphometry and number of villi in the jejunum part of small intestine in broiler chicken.

Key words: Broiler chicken, cow milk, fermentation, morphometry, probiotic, soymilk

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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

The small intestine provides a prime location for digestion and absorption of available nutrients. The important histological component in this portion is the villus[1]. The increase in villus length results in an enlargement of the contact surface with the nutrients and improves absorption[2], which may be linked to improved growth performance.

Probiotics contain lactic acid-producing bacteria that serve to improve the digestive and nutrient absorption processes. Probiotics can increase the activity of enzymes, i.e. sucrose, lactose, and tripeptidase in the small intestines. Probiotics providing from the starter period have been assumed to adopt the broilers to the probiotic microorganism and to help them to improve the balance of intestinal microflora. Probiotics can increase the activity of digestive enzymes, therefore the absorption of nutrients being optimized in line with the increasing area of absorption as probiotics can influence the intestinal anatomy like increased density and size of villi in the small intestine[3].

In this study fermented milk was used as a probiotic. Fermented milk used was soy milk and cow's milk. Soy is a group of oligosaccharides consisting of sucrose, stakiosa and raffinose that are hard to digest and to absorb in the intestine, however, it helps by acting as a growth substrate for useful bacteria in the intestine. Fermented soy milk also contains antioxidants other than probiotic bacteria like fermented soybeans[4]. Lactic acid bacteria in fermented soy milk has a role in improving the isoflavone digestibility[5]. Isoflavones are secondary metabolite compounds that are widely synthesized by plants. Amadou *et al*.[4] stated that in the soybean, isoflavone content ranges from 2 to 4 mg g^{-1}. Various types of isoflavones are genistein, daidzin and glisitin[6]. Antioxidants have been reported to have an effect to improve intestinal villi, which in turn have an impact on increased absorption. Fermentation of lactic acid bacteria also results in hydrolysis of soy protein into short peptides like Phe-Asp-His-Val-Glu and PheAsn-His-Leu-Asp-His, which is able to decrease DPPH free radicals, thus acting as antioxidants. Fermentation also causes isoflavone transformation into free isoflavone compounds, i.e aglycones which have higher antioxidant activity than in bound form[4].

Several studies have shown the benefit of probiotics on gut morphology and performance which suggest that by dietary means, it is possible to positively affect the development of the gut and to provide the competitive advantage in favor of beneficial bacteria which can alter not only gut dynamics but also many physiologic processes[7-9]. Therefore, the present study was carried out to evaluate the effect of fermented soy and cow milk as probiotic on small intestine morphometry of broiler chicken.

MATERIALS AND METHODS

Birds, housing and diets: A total of 100 day-old commercial broiler chicks were used in this study for the period of 45 days. The chicks were allocated to 4 treatment groups in a complete randomized design (CRD), Each treatment was replicated 04 times with 5 chicks in each replicate. The treatments consisted of birds fed: only basal feed (T0), basal feed with 100% fermented cow milk (T1), basal feed with fermented cow milk+fermented soy milk in 50:50% ratio (T2) and basal feed with fermented cow milk+fermented soy milk with a 75:25% ratio (T3). Chicks were housed in the litter cage system made from bamboo. The cage size was $70\times70\times70$ cm, each of which contained 5 chicks. The basal diets were provided in mash form with protein and energy content as 21.97% and 3011 kcal kg^{-1} respectively. Feed and water was provided *ad libitum*.

Processing of probiotic: Fermentation and assessment of cow and soy milk were done in the research laboratory of Faculty of Animal Husbandry, University of Padjadjaran. Fermentation of cow's milk and soybean milk was performed using *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum.* The fermented products were used as probiotics and fed to chicks using oral injection as desired doses.

Morphometry measurement: Following the slaughter of birds, jejunum samples were collected from the small intestine of broilers in each treatment group. The samples were washed in physiological NaCl, then fixed in Bouin for 2 days. The samples were dehydrated in alcohol with different concentrations for 30 min each i.e. 70, 80, 90 and 100%. Afterward, the samples were cleaned using xylol and alcohol 100% 3 times for 5 min each in 1:3, 1:1, 3:1 ratio. The samples were then infiltrated in an oven at 56°C using xylol and paraffin solutions in the ratio of 3:1. 1:1, 0:1 in sequences for 15 min. Samples were prepared using standard paraffin embedding procedures by sectioning at 6-8 μm thickness and kept for 1 day. The samples were then stained by Haematoxylin-Eosin. The sample was soaked in hematoxylin ehrlich for 2-10 min. HNO_3 0.5 % in 70 % ethanol was used to reduce the excess color. Alcohol and xylol were used to wash the samples. The samples were stained with eosin and thereafter hematoxylin washed alternately with 96% alcohol, 100% alcohol, alcohol:xylol and xylol I and II in sequences. In the end, the sample was covered with a mounting agent and viewed under the microscope.

Statistical analysis: The statistical analysis was performed to know the effect of treatment using one-way analysis of variance (ANOVA). Results obtained were presented as mean±standard error. The significant differences among different treatment means were investigated using Tukey's test by considering differences significant at $p<0.05$.

RESULTS AND DISCUSSION

The results of jejunal morphometry are presented in Table 1 and are depicted in Fig. 1-3. There was no significant ($p>0.05$) effect of using fermented cow or soy milk as probiotic in the diet of broiler chicken on jejunal villus height when compared with the control. Other researchers[10] also did not find positive effects of yeast supplementation on the jejunal villus height similar to the current study. The same result of no difference after probiotic supply within duodenal villus height was also reported earlier[11]. Pelicano *et al.*[3] proved no difference in villus height after *Bacillus subtilis* addition compared to the control group. Awad *et al.*[12] found no difference in duodenal villus height and width after synbiotic supply. Although, no effect on the improvement of villus height was observed with probiotic supplementation but other researchers have reported an improvement in the villus height by probiotic supplementation. Samli *et al.*[13] found significant differences in ileal villus height after probiotic (*Enterococcus faecium*) supplementation. However, Chichlowski *et al.*[14] reported that probiotic containing lactobacilli *Bifidobacterium thermophilum* and *Enterococcus faecium* increased the jejuna villus height compared with control. Samanya and Yamauchi[15] also reported longer villi in the ileum of adult male layers with a slight improvement in feed efficiency after dietary addition of *Bacillus subtilis* var. *natto*.

Further, in this study, there was a non-statistical ($p>0.05$) improvement in the jejunal villus width and a number of villi in chicken fed fermented cow or soy milk as probiotic in the diet when compared with the control. The highest improvement in the jujunal villus width and number of villi was found in the group wherein basal feed was supplemented with 100% fermented cow milk (T1) as probiotic. Jwher *et al.*[16] also reported positive effects on final body weight and morphometry in the jejunum in effective microbes supplemented groups in broiler chicken. Pelicano *et al.*[3] also reported the higher number of villi in the ileum and jejunum in broiler chickens fed with *B. subtilis* based probiotic.

Increasing the villus width and the number suggests an increased surface area capable of greater absorption of available nutrients. Increase in the villus surface area is directly correlated with increased epithelial cell turnover and activated cell mitosis[17]. It is understood that greater villus surface area is an indicator that the function of intestinal villi is activated[18]. It is assumed that an increased number and surface area of villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brush border enzymes and nutrient transport systems[19]. It is known that morphology of the

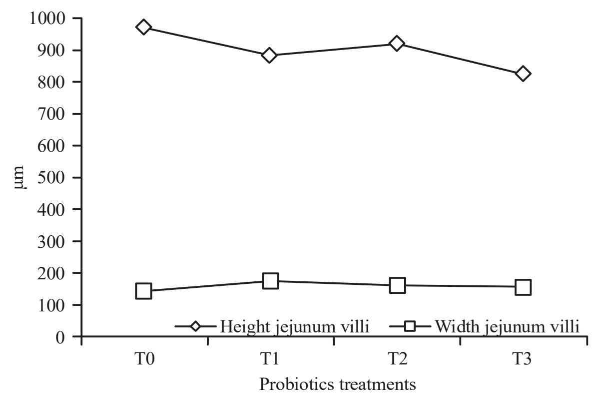

Fig. 1: Effect of probiotics based on fermentes cow and soy milk on height and width of jejunal villi of broiler chicken

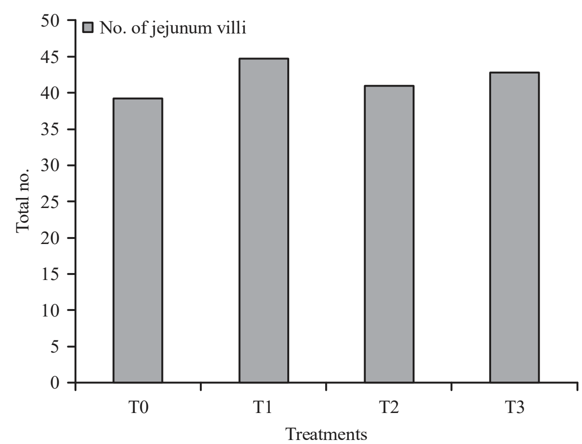

Fig. 2: Effect of probiotics based on fermentes cow and soy milk number of villi in Jejunum of broiler chicken

Table 1: Effect of Fermented cow milk and soymilk on jejunum morphometry in broiler chicks

	Treatments			
Parameters	T0	T1	T2	T3
Height of jejunum villi (μm)	972.81±108.10	883.02±74.51	921.19±89.95	825.26±44.79
Width of jejunum villi (μm)	144.96±19.12	174.72±22.38	160.63±17.03	154.98±22.93
No. of jejunum villi	39.40±5.18	44.80±2.68	41.20±4.21	43.00±2.92

Fig. 3: Morphometric visualization of jejunal villi fed probiotic based on fermented cow and soy milk

alimentary system, especially the structure of the villi in different parts of the gut, influences digestion and absorption of nutrients. Increased height and width of the villi increases the digestion and the absorption surface area, thereby increasing utilization of available nutrients[20]. The improvement in the morphometry and number of villi as achieved in present study in turn results in increased production performance of broiler chicken as reported by many researchers. Genetic progress pointed to higher final body mass, improved feed conversion due to improved gut morphology, increased villus surface area in fast-growing broilers[21]. Timmerman *et al.*[22] studied the effects of chicken-specific probiotics, consisting of 7 *Lactobacillus* species in broiler chickens and observed increased productivity in birds with probiotic supplementation compared to a control group. Gao *et al.*[10] also reported improved growth performance in broiler chickens, due to improved villus morphometry.

CONCLUSION

In conclusion, using a dietary fermented cow or soy milk in the diet resulted in improvement in the morphometry and the number of villi in the jejunum part of small intestine, especially fermented cow milk. This in turn would result in improved production performance of broiler chicken.

SIGNIFICANT STATEMENT

This study discovered that using fermented cow or soy milk as probiotic in the diet of broiler chicken is beneficial in improving the morphometry and the number of villi in a jejunal part of small intestine. This study will help the researchers to explore the effect of various combinations of fermented cow and soya milk as probiotic for ameliorating the gut morphology and the performance of broiler chicken. Thus a new theory on using fermented cow and soybean milk in the ration of broiler chicken may be arrived at.

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