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Original Article

Supplemental Effect of Lactobacillus Plantarum on the Growth Performance, Nutrient Digestibility, Gas Emission, Excreta Microbiota, and Meat Quality in Broilers

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ABSTRACT

The goal of this study was to investigate the effects of *Lactobacillus plantarum* (*L. plantarum)* supplementation on the growth performance, nutrient digestibility, gas emission, excreta microbiota, and meat quality in broilers. A total of two hundred eighty-eight, one-day-old Ross-308 chicks (mixed sex) were randomly allocated to one of two treatments with eight replicated cages (18 chicks per cage). For a period of 35 days, control treatment chicks were fed commercial corn-soybean meal-based (CON) mash form diet, whereas, LP (*Lactobacillus plantarum)* - chicks were fed CON with 0.10% *L. plantarum* supplement. The dietary inclusion of 0.10% *L. Plantarum* supplementation has a trend to increase the body weight gain (BWG) of broilers at day 7(p=0.079) and significantly increased at day 21 (p=0.011) and the overall trial period (p=0.037) compared to the CON diet. In addition, 0.10% *L. plantarum* supplementation to basal diet significantly decreased H_2S (p=0.046) concentration. Also, it has significantly increased the excreta *Lactobacillus* population (p=0.041) and reduced the *E. coli* count (p=0.054) compared to the CON diet. However, throughout the trial there were no significant differences observed on nutrient digestibility of dry matter, nitrogen, and gross energy, as well as meat quality traits in broilers, fed 0.10% *L. plantarum* diet. In summary, the inclusion of *L. plantarum* supplementation has a beneficial effect on growth performance, excreta microbiota, and gas emission. From this, we conclude that 0.10% of *L. plantarum* could be a potential feed additive to enhance poultry production.

INTRODUCTION

Poultry production has grown rapidly and becomes a competitive industry all over the world. The growth of poultry production has a profound impact on the demand for nutritious feed ingredients. Although feed addition plays an important role in livestock diet, the availability of low-cost and high-quality feeds is even more important if poultry production continues to grow quickly and to meet consumer demand for animal protein. On a large-scale rearing farm, chickens are exposed to a variety of diseases that leads to stressful situations and severe economic losses. To tackle this situation, the use of antibiotic growth promoters (AGP) has been increased. However, in the recent years, the haphazard use of AGP has raised concerns due to anti-microbial resistance. In response to this apparent threat, South Korean government has banned the use of AGP in livestock feed since 2011 (Sampath *et al.,* 2021a). Thus, manufactures and nutritionists were urged to seek alternative feed additives that could eventually stimulate the growth ability of broilers. One of the eco-friendly approaches to growth promotion involves the use of probiotics, which have been used in the livestock industry for decades (Smith, 2014).

Probiotics are defined as live microorganisms that are beneficial to the host (Upadhaya *et al.,* 2016). Probiotics have been shown to modulate intestinal microbiota, improve poultry performance, and reduce disease risk in the livestock industry (Abudabos *et al.*, 2015). Lactic acid bacteria (LAB) are widely distributed in the digestive tract of humans and animals (Shanmugam *et al.,* 2019). Besides, *L. plantarum* has probably been considered as the safest species and used in both human food and animal feed (Kanmani *et al.,* 2003). Moreover, it has the potential to improve metabolic activity and nutrient utilization by modulating the intestinal microbiota of broiler (Gao *et al.,* 2017). In addition, *L. plantarum* has been shown to promote the production performance, immune function, and intestinal microbiota homeostasis of broilers (Shen *et al.,* 2013). Also, metabolite combinations generated from *L. plantarum* have improved the fecal LAB population and small intestinal villus height by reducing fecal pH, and *E. coli* counts (Loh *et al.,* 2014). Yet, limited studies have been conducted testing the effects of 0.10% *L. plantarum* supplementation in broilers. Therefore, we hypothesized that supplementation of 0.10% *L. Plantarum* could potentially exert a benefit to broilers. Thus, the goal of this study was to analyze the effects of *L. plantarum* supplementation on the growth performance, nutrient digestibility, gas emission, excreta microbiota, and meat quality in broilers.

MATERIALS AND METHODS

This study was conducted at the poultry farming section located in Jeonui (Sejong, South Korea), pursuant to the approval (No: DK-1-2022) of the Animal care and use committee of Dankook University (Cheonan, Republic of Korea). Before starting the trial, rearing houses and the equipment were disinfected. A total of 288 Ross308 1-day-old chicks (hybrid) with an initial weight of 42.61 ± 0.49g (mean ± SD) were procured from Cherry-Buro hatchery (Cheonan, South Korea) and fostered in a complete randomized, multi-layer battery cage for 35-days with 8 replicates of 18 birds per cage with the pleasant environment of 33±1℃ room temperature for the first 3 days. Later the room temperature was slowly reduced up to 24℃ (60% humidity) and maintained so until the end of the trial.

The *L. plantarum* probiotic used in the experiment was obtained from Micro solution, Co Ltd, a commercial company located at Gwangju (South Korea). It contained 1.2 × 10^9 colony-forming units (CFU kg–1) of *L. plantarum*. The dietary treatment includes: CON (Basal diet); LP, (CON + 0.10% probiotic). A nutritious diet during the grower (d 1-21) and finisher (d22-35) stages were formulated to meet the requirements of NRC 1994 (Table 1). After the initial weighing of the basal diet, probiotic was incorporated in the feed at the prescribed level (0.10%) excluding the control group and provided to broilers for 35 days at the same time (14:00–15:00) whilst, clean water and feed was provided until the end of the experiment. Broilers were weighed at days 7, 21, and 35. The amount of diet consumed and remaining (each cage) were recorded daily to evaluate the feed intake (FI). On day 35, body weight (BW), feed intake (FI), feed conversion ratio (FCR), and the mortality rate were recorded. Chromic oxide (0.3%) as an indigestible marker was added to broiler diet on day 28 and provided for about one week until the end of the experiment to measure the nutrient digestibility. The representative feed samples were collected using the sterilized plastic bags from each treatment group right after mixing the marker.

Table 1 – Feed composition of broiler (as fed-basis).

Item	Grower	Finisher
Ingredients (%)		
Corn	48.55	55.35
Soybean meal	30.12	26.54
Corn gluten meal	13.00	10.00
Wheat bran	3.00	3.00
Soy oil	1.75	1.50
TCP	1.85	1.85
Limestone	0.91	0.92
Salt	0.36	0.36
Methionine (99%)	0.19	0.19
Lysine	0.07	0.09
Mineral mix[1]	0.10	0.10
Vitamin mix[2]	0.10	0.10
Total	100.00	100.00
Calculated value		
Crude protein, %	21.07	19.40
Ca, %	1.08	1.07
P, %	0.82	0.79
Available P, %	0.53	0.52
Lys, %	1.15	1.06
Met, %	0.52	0.50
ME, kcal/kg	3200	3200
FAT, %	4.54	4.34
Fiber, %	3.46	3.27
Ash, %	6.52	6.24

[1] Provided per kg of complete diet: 37.5 mg Zn (as $ZnSO_4$); 37.5 mg Mn (as MnO_2); 37.5 mg Fe (as $FeSO_4 \cdot 7H_2O$); 3.75 mg Cu (as $CuSO_4 \cdot 5H_2O$); 0.83 mg I (as KI); and 0.23 mg Se (as $Na_2SeO_3 \cdot 5H_2O$).

[2] Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 IU of vitamin E, 2.55 mg of vitamin K_3, 3 mg of Thiamin, 7.5 mg of Rivoflavin, 4.5 mg of vitamin B_6, 24 ug of vitamin B_{12}, 51 mg of Niacin, 1.5 mg of Folic acid, 0.2 mg of Biotin and 13.5 mg of Ca-Pantothenate.

On day 35, fresh excreta samples were randomly (32 birds/treatment) (4 birds/ cage) collected using a stainless steel collection tray. The excreta samples were pooled and transported to the laboratory, and stored at -20℃ to examine the nutrient digestibility of dry matter (DM), nitrogen (N), and energy (E). Prior to analysis, freeze-dried samples were placed in a digital hot air-drying convection oven at 105℃ for 24 hours. The samples were then taken out from the oven, milled, and sieved using a 1mm screen sieve. DM and N procedures were carried out according to the method of AOAC (2005). GE was analyzed using Parr 6400 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA), whereas N was analyzed using Tecator™ Kjeltec8400 analyzer (Hoeganaes, Sweden). The chromium absorption was identified using UV-1201 spectrophotometry. The total tract digestibility was calculated using: ATTD (%) = 100-[(NF/ND) ×(CrD/CrF)] ×100]. Hence NF, ND, CrD and CrF were referred as nutrient concentration in the excreta sample, nutrient concentration in the diet, chromium concentration in the diet, and chromium concentration in the excreta sample, respectively.

On day 35, excreta samples were collected (32 birds/ treatment) (4 birds/ cage) using a stainless steel tray, homogenized and stored in sterilized microtubes at 16:00 (pm), placed in an ice container, and immediately taken to the laboratory. 1gm of fresh excreta sample was taken and diluted in 9ml of 1% peptone solution and mixed using vortex mixer. The microbial analysis was done according to the procedure of Sampath *et al.* (2021b). Around 17:00 (pm) fresh excreta samples (approximately 300 g) were randomly collected from (32 birds/treatment) (4 birds/ cage) the pooled well, and stored in an airtight plastic box of 2.6 L with a slight hole on one side, fasten tightly with adhesive tape and fermented at 25℃ for 7 days. On the 8th day, a 100 ml sample was taken away from the headspace (2cm) for the air circulation, and the box was re-sealed. To know the crust formation on the surface the sample container was manually shaken for about 30 seconds. Finally, CO_2, acetic acid, H_2S, NH_3, and methyl mercaptans were measured using the scopes of 5.0 to 100.0 ppm (No. 3La, detector tube; Gastec Corp. Kanagawa, Japan) and 2.0 to 20.0 ppm (4LK, detector tube; Gastec Corp).

Broilers (32 birds/treatment) (4 birds/ cage) were sacrificed by cervical dislocation. The abdominal fat, liver, gizzard, spleen, bursa of fabricius, and breast muscle were carefully removed by the experts. The relative organs were weighed individually and estimated as mass BW. The respective samples were taken to the laboratory, and the breast meat was separated for meat quality analysis. The color parameters such as redness, lightness, and yellowness standards of each sample (surface) were measured at 3 locations with a portable Konica Minolta CR-400 chroma meter (Osaka, Japan). The pH, water holding capacity (WHC), drip loss, and cooking loss were calculated following the methods of Sampath *et al.* (2021b).

Statistical analysis

All the data were analyzed as a completely randomized block design using the GLM procedure of SAS (version 9.2; SAS Institute, Cary, NC). When significant differences were identified among treatment means, they were separated using T- test. The cages were considered as experimental units. Variability in the data was expressed as the standard error of means $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

L. plantarum was widely used as probiotics and exerts a beneficial effect on the host by modulating their intestinal tract (Cebeci *et al.*, 2003). The growth performance of broilers fed L. *plantarum* supplement is presented in Table 2. The dietary inclusion of 0.10% probiotics supplementation tends to increase (p=0.079) the BWG on the 7th day and significant increase on the 21st day (p=0.011) and the overall trial period (p=0.037) compared to the CON diet. Our result agreed with Peng *et al.*, (2016) who observed a higher ADG in broiler fed *L. plantarum* supplementation. Similarly, Kalavathy *et al.* (2003) reported that dietary *Lactobacillus* had improved the body weight gain of broilers. On the other hand, Ramarao *et al.* (2004) reported that the body weight gain of broilers was not influenced by dietary probiotic supplementation, which opposed to the findings of our experiment. Although Peng and co-authors report that the dietary inclusion of *L. plantarum* supplementation has improved the feed conversion ratio of broilers, in this study the broilers fed 0.10% *L. plantarum* supplement failed to improve FCR and the feed intake throughout the experimental period. To support our results, Siadati *et al.* (2017) reported that *Lactobacillus* supplement failed to influence the feed intake of Japanese quails. The reason for the improvements in body weight gain of broilers fed 0.10% *L. plantarum* in the current study was probably due to the increased population of beneficial intestinal bacteria and reduction of

the pathogenic bacteria residents. The provision of nutrient diet with particular energy and amino acids is more important for the efficient feed utilization (Yi *et al.,* 2015). Hence, we assume the lack of feed intake and FCR results might be due to the energy or protein content in the experimental diet or due to the environmental factors.

Table 2 – The effect of dietary L. *plantarum* supplementation on growth performance of broilers[1]

Items	CON	LP	SEM[2]	*p*-value
d 1 to 7				
BWG, g	136	140	2.04	0.079
FI, g	163	168	5.27	0.371
FCR	1.201	1.198	0.04	0.955
d 7 to 21				
BWG, g	630[b]	662[a]	10.86	0.011
FI, g	871	895	22.6	0.318
FCR	1.383	1.351	0.02	0.115
d 21 to 35				
BWG, g	906	934	34.19	0.416
FI, g	1957	1989	42.43	0.456
FCR	2.172	2.138	0.08	0.706
Overall				
BWG, g	1671[b]	1736[a]	28.34	0.037
FI, g	2991	3052	52.84	0.273
FCR	1.792	1.759	0.04	0.444
Mortality	4.86	6.25	2.54	0.594

[1]Abbreviation: CON (Basal diet); LP, CON + 0.10% probiotics.

[2]Standard error of means.

[a,b]Means in the same row with different superscripts differ ($p<0.05$).

A healthy intestinal tract is essential for livestock animals to absorb the nutrients and to provide a barrier against pathogenic bacteria (Hong *et al.*, 2005). Earlier studies have reported that *Lactobacilli* could exert a positive effect on the gastrointestinal tract as they increase feed consumption and nutrient absorption from the intestines (Awad *et al.*, 2009). Once, *Lactobacillus* supplements enter into the intestinal tract it may convert carbohydrates into lactic acid, reduce pH of the intestinal and increase the activities of trypsin, amylase, lipase, and total proteolytic enzymes to increase the length of villi and decrease the depth of the recess, which is beneficial for the digestion and absorption of food nutrients by the animals (Högberg and Lindberg, 2006). In the current study, broilers fed 0.10% *L. plantarum* supplementation failed to affect DM, N, and E (Table 3). Similarly, Chen *et al.* (2006) also noted that finishing pigs fed Bacillus-based probiotic supplement has no significant differences on digestibility of DM and N. Moreover, Shon *et al.* (2005) indicated that growing pigs fed 0.2% *Lactobacillus* complex (1×109 CFU/kg) diet did not affect the ATTD of DM and N. In contradiction, Liu *et al.* (2018) stated that probiotic (*B. subtilis and S. cerevisiae*) supplementation improved nutrient digestibility of growing pigs, while the similar effect was addressed by Jørgensen *et al.* (2016). We assume that the variation in these results may be due to different probiotic strains or due to the difference in animals. The lack of nutrient digestibility in broilers fed 0.10% *L. plantarum* is unknown, thus further studies are needed to clarify the effect of *L. plantarum* on broilers nutrient digestibility by altering the supplementation level of experimental diet composition.

Table 3 – Effect L. *plantarum* supplementation on nutrient digestibility of broilers[1]

Items	CON	LP	SEM[2]	*p*-value
Finish				
Dry matter	71.98	73.50	0.15	1.072
Nitrogen	69.94	70.64	0.51	1.051
Gross energy	70.95	72.12	0.36	1.224

[1]Abbreviation: CON (Basal diet); LP, CON + 0.10% probiotics.

[2]Standard error of means.

The harmful substances produced in excreta may lead to various health issues to the farmworkers as well as animals (Mcmichael *et al.*, 2007). In this study, broiler fed 0.10% probiotic supplementation has significantly decreased H_2S (p=0.046) emission, whereas it fails to affect other parameters such as NH_3, total mercaptans, CO2, and acetic acid (Table 4). Yet, our result was partially agreed with Zhao *et al.* (2015) who observed a decreased H_2S, NH_3, and total mercaptans emission in pigs fed *L. plantarum* and *L. reuteri* complex diet. Similarly, Han *et al.* (2005) reported that 0.2% of complex probiotic supplement which contains *Lactobacillus* had significantly decreased NH_3 emission in growing pigs. Previously, Chu *et al.* (2011) reported that the inclusion of probiotics in livestock feed has effectively decreased the level of ammonia, fecal pH, volatile organic matter and helps to get rid of the toxic odor. Apart from this, *Escherichia coli* is one of the most important bacteria for the cause of diarrhea

Table 4 – Effect L. *plantarum* supplementation on excreta gas emission in broilers[1]

Items, ppm	CON	LP	SEM[2]	*p*-value
Finish				
NH_3	14.7	12.5	3.08	0.507
H_2S	1.8	0.8[ab]	3.89	0.046
Methyl mercaptans	9.0	6.6	2.58	0.528
CO_2	1725	1425	340.9	0.412
Acetic acid	4.7	2.7	1.34	0.192

[1]Abbreviation: CON (Basal diet); LP, CON + 0.10% probiotics.

[2]Standard error of means.

[a,b] Means in the same row with different superscripts differ ($p>0.05$).

in animals. Previous studies have shown that *L. plantarum* diet could inhibit the growth of pathogens by competing with the limiting nutrients to regulate the composition of intestinal microflora and to form a biological barrier (Tian *et al.*, 2010). The present study reveals that the inclusion of 0.10% *L. plantarum* in broiler diet has significantly increased the *Lactobacillus* population (p=0.041) and reduced the *E. coli* counts (p=0.054) and no effect on *Salmonella* counts (Table 5). To support our results, Nguyen *et al.* (2019) stated that probiotics mixture (*B. coagulans, B. licheniformis, B. subtilis, and C. butyricum*) has significantly improved fecal *Lactobacillus* populations and decreased *E. coli* counts in weaning pigs. One probable reason for the increased BWG and decreased concentration of H_2S in the excreta emission may be due to the presence of good bacteria (*Lactobacillus*) in the gut of broilers.

Table 5 – Effect L. *plantarum* supplementation on excreta microbiota of broilers[1]

Items, log_{10}cfu/g	CON	LP	SEM[2]	*p*-value
Finish				
Lactobacillus	9.13	9.35 [ab]	0.05	0.041
E. coli	6.37	6.16 [ab]	0.05	0.054
Salmonella	4.38	4.28	0.121	0.417

[1]Abbreviation: CON (Basal diet); LP, CON + 0.10% probiotics.

[2]Standard error of means.

[a,b] Means in the same row with different superscripts differ (p<0.05).

The determination of poultry meat quality become a complicated concept since it depends on consumer preferences (Ishamri Ismail and Seon Tea Joo, 2017). Such meat quality traits were not affected by the experimental diet. Though, Kim *et al.* (2008) demonstrated that the inclusion of 0.1% complex probiotics which includes *L. plantarum* (1.0×10^8 cfu/g) in the diet of finishing pig has reduced the drip loss and increased meat color, however, in this study the meat color and drip loss in broilers fed 0.10% *L. plantarum* were not affected (Table 6). To date, the effect of supplementing 0.10% *L. plantarum* in broiler diets, on the meat quality is not well elucidated. Thus, enhance the comparisons could not be made with other studies. Further studies are needed with different doses of *L. plantarum* to evaluate their effects on the production traits of broilers.

CONCLUSION

Our data revealed that the administration of an *L. plantarum* strain could improve the BWG, reduced H_2S concentrations in the excreta thereby contributing to reduce the release of harmful gas emissions from poultry farms. In addition, the administration of 0.10% *L. plantarum* plays a vital role in the modulation of the gut microbiota as confirmed by the increase in excreta of *Lactobacillus* population and decrease in *E. coli* counts. From the obtained results, we concluded that 0.10% of *L. Plantarum* could be used as a potential feed additive to enhance the poultry production.

Table 6 – Effect L. *plantarium* supplementation on meat quality and organ weight of broilers[1]

Items	CON	LP	SEM[2]	*p*-value
Relative organ weight, %				
Breast muscle	18.04	18.14	1.09	0.925
Liver	2.52	2.61	0.16	0.621
Spleen	0.14	0.17	0.02	0.378
Abdominal fat	0.60	1.26	0.41	0.166
Bursa of Fabricius	0.16	0.13	0.03	0.292
Gizzard	1.92	1.72	0.11	0.309
Breast muscle color				
Lightness(L*)	57.52	57.83	1.51	0.845
Redness(a*)	11.76	11.55	0.78	0.799
Yellowness(b*)	12.19	13.60	2.1	0.531
pH value	5.72	5.62	0.05	0.095
Cooking loss, %	21.02	19.98	2.89	0.731
WHC, %	54.01	54.63	5.19	0.908
Drip loss, %				
d 1	3.97	3.04	0.75	0.261
d 3	6.77	6.19	0.82	0.507
d 5	12.66	14.31	0.92	0.124
d 7	17.64	16.83	0.59	0.218

[1]Abbreviation: CON (Basal diet);LP, CON + 0.10% probiotics.

[2]Standard error of means.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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