



# Evaluation of *Lactobacillus Plantarum* Additive on Growth Performance, Excreta Microbiota, Nutrient Digestibility, Gas Emission, and Meat Quality in Ross308-Broilers

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## ■ Keywords

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## ABSTRACT

The intention of this study was to analyze the effects of *Lactobacillus plantarum* (*L. plantarum*) additive with different nutrient density diets on growth performance, excreta microbiota, nutrient digestibility, gas emission, and meat quality in Ross308-broilers. A total of 576 mixed-sex, 1-d old Ross-308 chicks were randomly allocated to one of four treatment groups with 8 replication and 18 chicks/cage. For a period of 35 days, HD and LD group chicks were fed with commercial corn and soybean meal-based basal diet which contains high and low nutrient density diet, respectively. The other treatment groups LP1 and LP2 chicks were fed with LD+ 0.05% and 0.01 % of *L. plantarum*, respectively. During day 21 and the overall experimental period, the body weight gain of broilers significantly increased ( $p < 0.05$ ) in HD and *L. plantarum* groups compared to the LD group. On day 35, broilers fed *L. plantarum* additive had significantly increased ( $p < 0.05$ ) the nutrient digestibility of dry matter and nitrogen compared to those fed HD and LD diets. Moreover, dietary inclusion of *L. plantarum* additive had significantly increased ( $p < 0.05$ ) *lactobacillus* population and decreased ( $p > 0.05$ ) *E. coli* and ammonium emission. However, the meat quality traits were not affected by experimental diets. In conclusion, we infer that a low-density diet with 0.1% of *L. plantarum* additive could serve as an excellent alternative feed additive to enhance the performance of broilers.

## INTRODUCTION

Broiler plays a vital role in the commercial poultry sector. While comparing to the other meat producing animals, the modern broilers can grow faster to fulfil the customers protein requirements in the shortest time (Castonon, 2011). Also, broiler meat and eggs have become the cheapest animal protein source for human consumption and play an important role in enhancing the health status of humans (Rudra *et al.*, 2018). As such, broiler meat demand has been increasing due to the growing population. Earlier days antibiotic was widely used in livestock feed as a growth promoter (Ogle Maureen, 2013) however, the overuse of antibiotic causes a bacterial resistance in animals and creates an adverse effect on human health through food chain (Upadhaya *et al.*, 2016). Consequently, South Korean government has prohibited the administration of antibiotics in animals feed, since 2011 (Sampath *et al.*, 2021). Thus, animal nutritionist has focused their attention to find an alternative feed additive that could improve the growth performance, immunity, and prevent necrotic enteritis diseases in livestock animals. As a result, prebiotic, probiotics, plant-based additives, and organic acids were found to be eco-friendly alternatives. Among those the application of probiotics has been considered as one of the best alternative and practiced in poultry diet for many decades (Smith, 2014).



Probiotics are defined as live microorganisms that are beneficial to the host (Upadhyaya *et al.*, 2016). Besides, *Lactic acid bacteria* (LAB), has been frequently used in broiler diets due to their substantial role in maintaining the intestinal ecosystem (Shanmugam *et al.*, 2020) and stimulating the immune system of the host (Saarela *et al.*, 2003., Shanmugam *et al.*, 2021). The main anticipation of probiotics in poultry feed is to increase the feed intake, nutrient digestibility, as well as to maintain a healthy microbial inhabitant (Lima *et al.* 2016). Moreover, *L. plantarum* probiotic strain has been considered as the safest species and used in both human and animal feed (Kanmani *et al.*, 2003). In 2019, Qiao *et al.*, reported that the administration of *L. plantarum* plays a dynamic role in modulating the gut microbiota of broilers. Also, Mountzouris *et al.* (2009) stated that probiotic supplement has increased the body weight and feed conversion ratio as well as reduced the mortality rate in broilers. On the other hand, Watkins & Kratzer (1983) stated that broilers fed *Lactobacillus* strains had reduced the numbers of coliforms in cecal microbiota. Although previous researches conducted with *L. plantarum* showed an enhanced production performance, immune function, and intestinal microbiota of broilers (Shen *et al.*, 2013) yet to the best of our knowledge limited studies were conducted testing the graded level of *L. plantarum* with different nutrient density diet on broiler performance. Due to the high cost of a high-density diet in animal nutrition, in this study, we used a low-density diet with probiotic (*L. plantarum*) at an affordable price. Moreover, we hypothesized that supplementation of a low-density diet with graded level of *L. plantarum* could be beneficial for enhancing the body weight, nutrient digestibility, *Lactobacillus* counts and reduce NH<sub>3</sub> odor in broilers. Therefore, the intention of this study was to analyze the effects of graded level of *L. plantarum* additive with different nutrient density diets on growth performance, nutrient digestibility, excreta microbiota, gas emission, and meat quality of Ross308-broilers

## **MATERIALS AND METHODS**

### **Ethical endorsement**

This experiment was conducted at Dankook University "Poultry farming unit" (Jeonui, Sejong, South Korea) in strict accordance with the guidelines of the Institutional Animal Care and Use Committee. Prior to the trial, the research protocols were revised and permitted (Permit No: DK-1-2022) by the Ethics Committee of Dankook University, South Korea.

### **Broiler Husbandry**

Before starting the trial, all equipment and rearing houses were disinfected. A total of 576 Ross308 a day-old Ross 308-broilers (mixed sex) with an initial average weight of 42.23 ± 0.05 g (mean ± SD) were obtained from the commercial hatchery Cherry-Buro (Cheonan, Korea). On the day of arrival, all chicks were weighed, distributed in multi-layer battery cages, and fostered for 35 days. First, the room temperature was maintained at 33 ± 1°C and gradually reduced to 24°C (60% humidity) and maintained throughout the trial. To maintain a hygienic environment rearing house was cleaned every week until the end of the study.

### **Feeds and Feeding programs**

Our experiment lasted for 35 days. The feeding program consisted of: starter [0-7 days], grower [8-21 days], and finisher [21-35 days]. The broilers were allotted to one of four dietary treatments with 8 replications and 18 chicks /cage, and the dietary treatments were: High-density (HD) and low-density (LD) which has corn-soybean meal based basal diet (CON) and no *L. plantarum* additive, whereas LP1 and LP2 were incorporated with – CON 0.05% and 0.1% *L. plantarum* additive. Basal diets (mash form) were formulated according to the recommended level of NRC 1994 (Table 1). *L. plantarum* additive used in the experiment was commercially purchased from micro solution, Co Ltd, located at Gwangju (South Korea). It contained 1.2 × 10<sup>9</sup> colony-forming units (CFU kg<sup>-1</sup>) of *L. plantarum*. The experimental diet was mixed in broilers feed at the prescribed level and provided for 35 days at the same time 14:00–15:00. Chicks had free access to clean water and feed until the end of the experiment.

### **Sample collection and Laboratory Analysis**

#### **Growth performance**

On days 7, 21, and 35 the body weight gain (BWG) of broilers were weighed using an electrical weight machine with minimum accuracy of ±1 g. The amount of diet consumed and leftovers were recorded on cage basis in the same time points. Following the BWG (per g), the feed intake (FI/g), feed to gain ratio (FCR-g /g), and the mortality rate was also recorded.

#### **Nutrient Digestibility**

Chromic oxide (0.3%) as an indigestible marker was added to the broiler diet on day 28 and provided for about one week until the end of the experiment to measure the nutrient digestibility. The representative



**Table 1** – Ingredient and chemical composition of experimental diets (as fed-basis).

Item	Starter		Grower	Finisher	
	HD	HD	LD (-2% CP)	HD	LD (-3% CP)
Ingredients (%)					
Corn	43.63	47.45	48.55	53.78	55.35
Soybean meal	35.08	31.28	29.28	28.18	25.18
Corn gluten meal	13	13	13	10	10
Wheat bran	3	3	3	3	3
Soyoil	1.76	1.74	1.75	1.51	1.5
TCP	1.81	1.81	1.85	1.81	1.85
Limestone	0.94	0.94	0.91	0.94	0.92
Salt	0.36	0.36	0.36	0.36	0.36
Methionine (99%)	0.19	0.19	0.19	0.19	0.19
Lysine	0.03	0.03	0.07	0.03	0.09
Mineral mix <sup>1</sup>	0.1	0.1	0.1	0.1	0.1
Vitamin mix <sup>2</sup>	0.1	0.1	0.1	0.1	0.1
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>		
Calculated value					
Crude protein, %	23	21.5	19.5	20	17
Ca, %	1.1	1.08	1.08	1.07	1.07
P, %	0.83	0.82	0.82	0.79	0.79
Available P, %	0.54	0.53	0.53	0.52	0.52
Lys, %	1.26	1.15	1.15	1.06	1.06
Met, %	0.54	0.52	0.52	0.5	0.5
ME, kcal/kg	3200	3200	3200	3200	3200
FAT, %	4.45	4.51	4.54	4.32	4.34
Fiber, %	3.55	3.48	3.45	3.30	3.27
Ash, %	6.76	6.57	6.52	6.30	6.24

<sup>1</sup> Provided per kg of complete diet: 37.5 mg Zn (as ZnSO<sub>4</sub>); 37.5 mg Mn (as MnO<sub>2</sub>); 37.5 mg Fe (as FeSO<sub>4</sub>·7H<sub>2</sub>O); 3.75 mg Cu (as CuSO<sub>4</sub>·5H<sub>2</sub>O); 0.83 mg I (as KI); and 0.23 mg Se (as Na<sub>2</sub>SeO<sub>3</sub>·5H<sub>2</sub>O). <sup>2</sup> Provided per kg of complete diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D<sub>3</sub>, 37.5 IU of vitamin E, 2.55 mg of vitamin K<sub>3</sub>, 3 mg of Thiamin, 7.5 mg of Riboflavin, 4.5 mg of vitamin B<sub>6</sub>, 24 ug of vitamin B<sub>12</sub>, 51 mg of Niacin, 1.5 mg of Folic acid, 0.2 mg of Biotin and 13.5 mg of Ca-Pantothenate.

feed samples were collected using the sterilized plastic bags from each treatment group right after mixing the marker. On day 35, approximately 50g fresh excreta samples were collected from 2 cages/treatment (32 birds/treatment) using stainless steel collection tray and homogenized. Then excreta samples were taken to the laboratory within 30 minutes, and stored at -20 °C to examine the nutrient digestibility of dry matter (DM), nitrogen (N), and energy (E). Prior to analysis, all feces and feed samples were placed in a hot air-drying convection oven at 105 °C for one day. Then the samples were grounded to pass 1mm screen sieve mesh. DM, N, and GE procedures were carried out according to the procedure of AOAC (2007). The chromium absorption was identified using UV-1201 spectrophotometry. GE was analyzed using Parr 6400 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA) and N was analyzed using Tecator™ Kjeltec8400 analyzer (Hoeganaes, Sweden). 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.

### **Microbial shedding**

On day 35, fresh cecal samples (deposited within 1hr) were collected from 32 birds/treatment (2cages/ treatment) using sterilized microtubes at 15:00 (pm), placed in an ice container, and taken to the laboratory within 30 minutes. To count the presence of microbes, 1gm excreta sample was taken and diluted (10-fold dilutions) with 9ml of 1% peptone solution and mixed using a vortex mixer. Then 0.02% of peptone solution was given into *Lactobacilli medium III*, *MacConkey*, and *Salmonella-Shigella* agar plates, respectively. Finally, the colony formations were enumerated by the methods of Sampath *et al.* (2021) and the results werelog<sup>10</sup> transformed for statistical analysis.

### **Gas Emission**

At the end of the experiment, fresh excreta samples (approximately 300 g) were collected from (2 cages/ treatment) around 17:00 (pm) pooled well, and stored



in an airtight container (2.6 L) which has a slight hole on one side, fasten tightly with adhesive tape and fermented at 25 °C for 7 days. On the 8th day, a 100 ml sample was taken away from the headspace (2cm) for 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<sub>2</sub>, acetic acid, H<sub>2</sub>S, NH<sub>3</sub>, and methyl mercaptans were measured using the methods of Nguyen and Kim. (2020).

### Meat Quality

On day 35, 36 birds/treatment were taken to the slaughter house and killed by cervical dislocation. The abdominal fat, liver, gizzard, spleen, bursa of fabricius, and breast muscle were cautiously removed by the experts. The relative organs were weighed individually and estimated as mass BW. The respective samples were taken to the laboratory, and 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). To determine the water holding capacity (WHC), 0.4g sample was placed in 125 mm diameter filter paper and pressed for about 4 min at 26 °C. Then the areas of the compressed sample and the expressed humidity were defined and determined by using a digitalized area-line sensor (MT-

10S, M.T. Precision Co. Ltd., Tokyo, Japan). The ratio of water: meat area was then calculated as WHC. The pH of the breast meat sample was measured using (T-bar) Testo 205- portable pH meter (Co. Ltd., USA) while, drip loss and cooking loss was calculated using the methods of Honikel (1998) and Sullivan *et al.* (2007), respectively.

### Statistical Analysis

By using the GLM procedure of SAS (Inst. Inc., Cary, NC, USA: SAS 2012) all data were analyzed in a complete randomized design. The cage served as the experimental unit for growth performance, nutrient digestibility, microbial counts and gas emission whereas for meat analysis individual bird served as experimental unit Prior to statistical analysis, microbial colony data were log-transformed. Orthogonal contrasts used to separate treatment: HD vs LD, HD vs LP1, 2, and LD vs LP1, 2. Variability in the data was expressed as the standard error of means. The probability values of 0.05 and 0.1 were considered as significant and trends, respectively.

## RESULTS

### Growth performance

The effect of *L. plantarum* additive with different nutrient density diets on the growth performance of broilers is shown in Table 2. During day 21 and the overall

**Table 2** – The effect of *L. plantarum* additive with different nutrient density diet on growth performance of broiler<sup>1</sup>

Items	HD	LD	LP1	LP2	SEM <sup>2</sup>	p-value		
						HD vs LD	HD vs LP1,2	LD vs LP1,2
d 1 to 7								
BWG, g	140	136	137	139	2	0.100	0.420	0.257
FI, g	164	163	163	165	4	0.824	0.969	0.789
FCR	1.175	1.201	1.192	1.189	0.028	0.547	0.670	0.785
d 7 to 21								
BWG, g	641 <sup>ab</sup>	630 <sup>b</sup>	653 <sup>ab</sup>	661 <sup>a</sup>	8	0.041	0.073	0.025
FI, g	897	871	880	884	13	0.179	0.359	0.512
FCR	1.349	1.383	1.349	1.380	0.018	0.235	0.526	0.452
d 21 to 35								
BWG, g	944	906	923	919	22	0.240	0.407	0.588
FI, g	1991	1957	1981	1971	31	0.452	0.707	0.619
FCR	2.110	2.172	2.155	2.149	0.051	0.361	0.477	0.738
Overall								
BWG, g	1646 <sup>ab</sup>	1571 <sup>b</sup>	1712 <sup>ab</sup>	1799 <sup>a</sup>	19	0.034	0.101	0.018
FI, g	3052	2991	3024	3020	33	0.197	0.462	0.438
FCR	1.745	1.792	1.767	1.779	0.024	0.157	0.319	0.507
Mortality	4.17	4.86	4.86	5.56	-			

<sup>1</sup>Abbreviation: HD, Basal diet -CON (high CP); LD, CON (low CP); LP1, LD + 0.05% *L. plantarum*; LP2, LD + 0.10% *L. plantarum*.

<sup>2</sup>Standard error of means.

<sup>a,b</sup>Means in the same row with different superscripts differ ( $p < 0.05$ ).



experimental period, the body weight gain (BWG) of the broilers were significantly increased ( $p=0.041$  and  $p=0.034$ ) with the HD diet. Whereas, compared to the HD diet the graded level of LP1 and LP2 additive has a trend to increase the BW ( $p=0.073$ ) of broilers. Also, the graded level *L. plantarum* additive has significantly increased the BW ( $p=0.025$  and  $p=0.018$ ) of broilers at day 21 and the overall trial period compared to those fed LD diet. Growth performance parameters excluding the BW, there was no difference observed on FI, FCR, and mortality of broilers throughout the experimental period.

### Nutrient Digestibility

The effect of *L. plantarum* additive with different nutrient density diets on nutrient digestibility of broilers is presented in Table 3. At the end of the experiment, DM digestibility was significantly increased ( $p=0.046$ ) in broilers fed HD diet compared to those fed LD diet. Moreover, broiler fed a diet containing increasing level *L. plantarum* additive has increased ( $p=0.038$ ) the dry matter digestibility compared to those fed LD diet. Also, the graded level of *L. plantarum* supplement has significantly increased ( $p=0.047$ ) the nutrient digestibility of N compared to those fed HD diet. In

**Table 3** – The effect of *L. plantarum* additive with different nutrient density diet on nutrient digestibility of broiler<sup>s1</sup>

Items, %	HD	LD	LP1	LP2	SEM <sup>2</sup>	p-value		
						HD vs LD	HD vs LP1,2	LD vs LP1,2
Finish								
Dry matter	72.79	71.98	73.13	73.98	1.06	0.046	0.171	0.038
Nitrogen	70.76	69.94	71.28	72.31	1.04	0.079	0.047	0.027
Gross energy	71.88	70.95	70.98	71.43	1.04	0.524	0.593	0.839

<sup>1</sup>Abbreviation: HD, Basal diet -CON (high CP); LD, CON (low CP); LP1, LD + 0.05% *L. plantarum*; LP2, LD + 0.10% *L. plantarum*.

<sup>2</sup>Standard error of means.

addition, broilers fed a low-density diet with the increasing level of *L. plantarum* additive has highly increased ( $p=0.027$ ) the N compared to those fed low density diet. Throughout the trial gross energy (GE) was not affected by the experimental diets which contains HD, LD, LP1, and LP2.

### Microbial shedding

Dietary inclusion of *L. plantarum* additive has significantly increased ( $p=0.05$ ) the *lactobacillus* population compared to those fed HD and LD diets.

At the end of the trial, broilers fed diet containing *L. plantarum* additive has trend ( $p=0.090$ ) to a significant decrease ( $p=0.041$ ) *Escherichia coli* counts compared to those fed HD and LD diet. *Salmonella* counts was not affected either by HD and LD or by *L. plantarum* additive (Table 4).

### Gas Emission

Allow nutrient density diet with an increased level of *L. plantarum* supplement has significantly reduced ( $p=0.035$ ) NH<sub>3</sub> and tend to decrease ( $p=0.076$ ) H<sub>2</sub>S

**Table 4** – The effect of *L. plantarum* additive with different nutrient density diet on excreta microbiota in broilers<sup>1</sup>

Items, log <sub>10</sub> cfu/g	HD	LD	LP1	LP2	SEM <sup>2</sup>	p-value		
						HD vs LD	HD vs LP1,2	LD vs LP1,2
Finish								
<i>Lactobacillus</i>	9.17	9.13	9.23	9.31	0.04	0.587	0.058	0.024
<i>E. coli</i>	6.17	6.27	6.10	5.18	0.05	0.171	0.090	0.041
<i>Salmonella</i>	4.31	4.38	4.37	4.35	0.07	0.435	0.530	0.781

<sup>1</sup>Abbreviation: HD, Basal diet -CON (high CP); LD, CON (low CP); LP1, LD + 0.05% *L. plantarum*; LP2, LD + 0.10% *L. plantarum*.

<sup>2</sup>Standard error of means.

emission in broilers. However, there was no difference observed on methyl mercaptans, CO<sub>2</sub>, and acetic acid throughout the trial (Table 5).

### Meat Quality

The addition of *L. plantarum* additive with different nutrient density diet on meat quality of broilers is

illustrated in Table 6. At the end of the study, broiler meat quality was not affected either by HD, LD diets, or by increased level of *L. plantarum* additive.

## DISCUSSION

Many studies have investigated the the effects of probiotics on the performance of monogastric animals



**Table 5** – The effect of *L. plantarum* additive with different nutrient density diet on gas emission of broilers<sup>1</sup>

Items, ppm	HD	LD	LP1	LP2	SEM <sup>2</sup>	p-value		
						HD vs LD	HD vs LP1,2	LD vs LP1,2
Finish								
NH <sub>3</sub>	13.5	14.7	13.1	12.1	2.2	0.656	0.417	0.035
H <sub>2</sub> S	1.4	1.8	1.1	0.8	0.3	0.397	0.332	0.076
Methyl mercaptans	8.5	9.0	7.4	3.8	2.0	0.862	0.312	0.234
CO <sub>2</sub>	1650	1725	1525	1450	236	0.828	0.589	0.435
Acetic acid	4.5	4.7	3.5	2.8	0.8	0.897	0.201	0.160

<sup>1</sup>Abbreviation: HD, Basal diet -CON (high CP); LD, CON (low CP); LP1, LD + 0.05% *L. plantarum*; LP2, LD + 0.10% *L. plantarum*.

<sup>2</sup>Standard error of means.

with a different strain (Suresh Kumar *et al.*, 2020; Balasubramanian *et al.*, 2018) however, only a limited amount of research has been performed using *L. plantarum* additive especially in broilers. Over the past few years, nutritionist attention has been focused on the application of *L. plantarum* in commercial poultry activities. As a result, Gao *et al.* (2017) study report that *L. plantarum* P-8 strain had a potential to improve the nutrient utilization and metabolic activity by modulating the intestinal microbiota of broiler chickens. Also, Ding *et al.* (2017) research noted that dietary supplemented with *Lactobacillus has* improved feed efficiency of hens. Similarly, Peng and co-authors (2016) reported that dietary *L. plantarum* B1 supplement has improved the feed conversion ratio of broilers on finisher period (day 42). Furthermore, Mohammadreza *et al.* (2015) stated that graded level of multi-strain probiotic (*L.*

*plantarum*, *L. bulgaricus*, *L. acidophilus*, etc) has linearly increased the BW and FCR of broilers was partially agreed with our findings as increased BWG. The reason for the improvements in body weight gain of broilers fed 0.1% *L. plantarum* in the current study was probably due to the increased population of beneficial intestinal bacteria and reduction of the pathogenic bacterial residents. However, excluding the BW, the FI, and FCR in this current study showed no significant differences ( $p>0.05$ ), which suggests that the average manufacturer's recommended level of *L. plantarum* additive might not be appropriate to enhance the productive results. Moreover, Yi *et al.* (2015) stated that the provision of a nutrient diet with particular energy and amino acids is the more important factor for effective feed utilization. Thus, we assume that the lack of feed intake and FCR

**Table 6** – The effect of *L. plantarum* additive with different nutrient density diet on meat quality of broilers<sup>1</sup>

Items	HD	LD	LP1	LP2	SEM <sup>2</sup>	p-value		
						HD vs LD	HD vs LP1,2	LD vs LP1,2
Relative organ weight, %								
Breast muscle	18.28	18.04	18.06	18.07	0.68	0.831	0.829	0.975
Liver	2.79	2.52	2.59	2.65	0.15	0.225	0.355	0.609
Spleen	0.15	0.14	0.14	0.16	0.02	0.854	0.957	0.873
Abdominal fat	0.88	0.60	0.86	0.97	0.28	0.429	0.896	0.304
Bursa of Fabricius	0.16	0.16	0.17	0.15	0.02	0.905	0.913	0.971
Gizzard	1.95	1.92	1.81	1.98	0.06	0.767	0.477	0.707
Breast muscle color								
Lightness(L*)	58.43	57.52	56.24	55.58	0.85	0.483	0.413	0.167
Redness(a*)	11.69	11.76	12.26	12.81	0.44	0.906	0.105	0.131
Yellowness(b*)	11.72	12.19	12.98	10.64	0.97	0.598	0.913	0.616
pH value	5.72	5.72	5.70	5.64	0.05	0.948	0.435	0.476
Cooking loss, %	19.00	21.02	18.90	19.14	1.89	0.474	0.990	0.414
WHC, %	55.80	54.01	52.79	53.55	3.69	0.722	0.550	0.848
Drip loss, %								
d 1	4.96	3.97	3.14	3.63	0.74	0.385	0.127	0.547
d 3	6.32	6.77	7.27	6.61	0.48	0.559	0.360	0.797
d 5	13.83	12.66	13.39	12.87	0.68	0.262	0.427	0.597
d 7	16.88	17.64	16.85	17.89	0.42	0.233	0.370	0.606

<sup>1</sup>Abbreviation: HD, Basal diet -CON (high CP); LD, CON (low CP); LP1, LD + 0.05% *L. plantarum*; LP2, LD + 0.10% *L. plantarum*.

<sup>2</sup>Standard error of means.



might be due to the energy or protein content in the experimental diet or maybe depends on other factors including the probiotic strains, ages of the animals, or the method of probiotic administration.

A study by, Awad *et al.* (2009) reported that *Lactobacilli* supplement has exerted a positive effect on the gastrointestinal tract by increasing the FI and nutrient absorption from the intestine. Similarly, Hong *et al.* (2005) stated that a healthy gut can easily absorb the nutrients and fight against the pathogenic bacteria in animals. Moreover, reducing the intestinal damage caused by pathogenic bacteria is of a great deal to improve the performance of broilers. In this study, the graded level of *L. plantarum* has significantly improved the nutrient digestibility of DM and N is consistent with previously published findings of Apata (2008) and Li *et al.* (2008) who noted a higher DM digestibility in broilers fed probiotic supplement. To support our results, Mountzouris *et al.* (2010) also stated that *Lactobacillus sp.*  $1 \times 10^8$  CFU/g probiotics supplementation has increased DM digestibility in broilers. The probable reason for the increased nutrient digestibility DM and N might be due to an increased population of *Lactobacillus* bacteria.

In previous reports, Hinton *et al.* (1992) reported that lactic acid bacteria have the ability to reduce the growth of harmful bacteria in the intestine. On the other hand, Watkins & Kratzer. (1993) reported that the supplement of *Lactobacillus* has fortified the beneficial intestinal microorganism and suppress the growth of coliforms bacteria in chicks. Compared to this result, our findings were similar as increased *lactobacillus* and decreased *E. coli* counts in broilers fed *L. plantarum* supplement. Our findings indicate that an increased level of *L. plantarum* may help the broilers to maintain their health status and to improve nutrient digestibility. The noxious gas emission is always connected to the nutrient utilization and intestinal microbial ecosystem (Hakkinen & Schneitz, 1996). Besides,  $\text{NH}_3$  gas released from the poultry farm causes major air pollution and that leads to serious health issues (Mc Michael *et al.* 2007). In 2011, Chu *et al.*, report that the addition of probiotics in livestock feed has effectively decreased the toxic odor like  $\text{NH}_3$ . Similarly, in this study broilers fed 0.05% to 0.1% of *L. plantarum* additive have effectively decrease  $\text{NH}_3$  and  $\text{H}_2\text{S}$  emission was correlated with previously published findings of Hassan and Ryu. (2012) who observed a reduced  $\text{NH}_3$  in broilers fed a diet containing probiotics. The main reason for decreased  $\text{NH}_3$  and  $\text{H}_2\text{S}$  odor in broilers may be due to increased nutrient digestibility and/or a healthy intestinal microbial ecosystem.

Determining the quality of chicken meat is a challenging task as it always depends on consumer perception of meat freshness (color) and quality (Ishamri Ismail & Seon Tea Joo, 2017). Moreover, the pH of meat become an important index to evaluate the quality of meat and it is closely connected to WHC. In 2016, Abdullah *et al.* reported that the influence of probiotic contains *Bacillus subtilis* strain has significantly decreased the pH of broilers meat. In addition, Astuti (2015) stated that broilers fed probiotics (LAB) supplementation has significantly reduced the cholesterol content of meat. However, in this study broilers fed graded levels of *L. plantarum* additive showed no impact on meat quality indexes. Yet, broilers fed a low-density diet with *L. plantarum* supplement attains a better meat quality is not presented. Thus, adequate comparisons could not be made. Moreover, our research team has planned to conduct further studies to know the exact cause for the lack of meat quality traits in broilers.

## CONCLUSION

Our data revealed that the administration of a low-density diet with *L. plantarum* strain could improve the BWG, nutrient digestibility of dry matter, and nitrogen. Also, it plays a vital role in modulating the gut microbiota as confirmed by the increase in *Lactobacillus* population and decrease in *E. coli* counts. Moreover, the graded level of *L. plantarum* additive with low- density diet has reduced  $\text{NH}_3$  and  $\text{H}_2\text{S}$  toxic odor emission from a poultry farm. Therefore, we infer that a low-density diet with 0.1% of *L. plantarum* would be cost-effective and an excellent alternative feed additive to enhance the performance of broilers.

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## DISCLOSURE

No potential conflict of interest was filed to this article.



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