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Addition of soybean meal extract with *Lactobacillus plantarum* in rations on protein digestibility and performance of broiler chickens

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ABSTRACT

This study examined the effect of adding soybean meal extract (SME) and *Lactobacillus plantarum* (LP) on protein digestibility and performance of broiler chickens. The material used was 8-day-old Cobb CP 707 strain broilers with a body weight of 137.89 ± 3.7 g. This study used a completely randomized design with 6 treatments and 4 replicates, with each replicate of 8 birds. The treatments were T0: basal diet (control), T1: basal diet + LP 1.2%, T2: basal diet + SME 0.15%, T3: basal diet + SME 0.30%, T4: basal diet + SME 0.15% + LP 1.2%, T5: basal ration + SME 0.30% + LP 1.2%. Parameters measured were the performance of broiler chickens, lactic acid bacteria population, *Escherichia coli* population, intestinal pH, protein consumption, protein digestibility, and antioxidant activity. The results showed that the addition of SME and *Lactobacillus plantarum* in the ration had a significant effect ($P < 0.05$) on total daily weight gain, total daily feed intake, total feed conversion, lactic acid bacteria population, *Escherichia coli* population, intestinal pH, protein consumption, protein digestibility, and antioxidant activity. Significantly higher total daily weight gains were observed in T5 (54.09 g) compared to T3 (51.27 g), T2 (46.98 g), T1 (46.64 g), and T0 (45.56 g). Total daily feed intake of T5 (79.94 g) was significantly higher those of T2 (74.52 g), T1 (74.17 g), and T0 (74.89 g). Feed conversion ratio was significantly lower in T5 (1.48) compared to the others, but not different from T4 (1.54). The LAB population of T5 (10.26 log cfu/g) was significantly higher than those of T2 (8.98 log cfu/g), T1 (8.99 log cfu/g), and T0 (6.99 log cfu/g). The *Escherichia coli* population of T5 (1.50 log cfu/g) was significantly lower than T1 (2.40 log cfu/g) and T0 (4.54 log cfu/g). Intestinal pH of T5 (5.90) was significantly lower than the others, but not different from T4 (5.91). Protein consumption of T5 (17.14 g) was significantly higher than T2 (15.98 g), T1 (15.90 g), and T0 (16.00 g), and protein digestibility of T5 (88.53%) was significantly higher than T2 (85.42%), T1 (82.92%), and T0 (80.37%). Malondialdehyde of T5 (0.80 nmol/ml) was significantly lower than T2 (1.01 nmol/ml), T1 (1.03 nmol/ml), and T0 (1.27 nmol/ml). Superoxide dismutase of T5 (21.43 U/ml) was significantly higher than all treatments. The study concludes that adding soybean meal extract with 0.30% and *Lactobacillus plantarum* 1,2% (T5) was effective in increasing protein digestibility and optimizing performance in broiler chickens.

Keywords: Broiler, Lactobacillus, Oligosaccharides, Soybean, Synbiotic

INTRODUCTION

Broiler chicken is a poultry livestock with a high efficiency in converting rations into meat. The benefits of broiler chickens include their ability to proliferate swiftly and generate meat (Alimuddin *et al*., 2021). Gous (2018) reports that broiler chickens with the genotype grow faster and need balanced nutrition to maximize their genetic potential. Public consumption of chicken meat has increased over time. Based on the Statistics Indonesia's Susenas (National Socio-Economic Survey) processed by Pusdatin, chicken meat consumption in Indonesia in 2019 was 5.70 kg/capita/year, while in 2021, chicken meat consumption increased to 6.55 kg/capita/year. This growth in demand, however, has not been matched by an improvement in quality, particularly in terms of food safety and health. The productivity and quality of products produced by broiler chickens are supported by providing rations with complete, balanced, and quality nutrients. One solution to support increasing broiler chicken productivity is to use prebiotics derived from natural ingredients and probiotics.

Soybean meal is a by-product of soybean processing that contains soybean oligosaccharides (SOS), so it can potentially be used as a prebiotic (Krismaputri *et al*., 2016[a]). Soybean oligosaccharides from soybean meal extract are one of the natural compounds that are friendly to consumer health because they do not carry residues into the resulting broiler chicken products (Suthama *et al*., 2018). The oligosaccharides in soybean meal extract include raffinose 0.73 g/100 g and stachyose 0.90 g/100 g (Krismaputri *et al.,* 2016[a]). The addition of soybean oligosaccharides (SOS) can trigger the growth of lactic acid bacteria. Results from Krismaputri *et al.* (2016[b]) show that giving SOS prebiotics from EBK at high levels effectively reduces the *E. coli* population in the digestive tract of chickens. Krismaputri *et al*. (2016[a]) added that this situation positively affects the existence of beneficial bacteria such as lactic acid (LAB) in the digestive tract of chickens. Soybean meal oligosaccharides can be utilized by the cecal microbial community of broiler chickens (Lan *et al*., 2007). Stachyose and raffinose, as the main components of oligosaccharides found in soybean meal, prevent, and inhibit the colonization of intestinal pathogenic bacteria and increase intestinal development and health (Zhu *et al.,* 2020). Oligosaccharides that undergo raffinose-derived modifications are resistant to the pH of the digestive tract and digestion by digestive enzymes, specifically α-galactosidase (Cresci *et al*., 1999).

Lactobacillus plantarum is a microbial culture of the LAB class, which can suppress the development of pathogenic microbes, lower pH, and activate digestive enzymes. *Lactobacillus plantarum* in vitro exhibits positive features such as antibacterial activity, acid tolerance, and good resistance to bile salts, allowing it to be employed as a probiotic (Belicová *et al*., 2013). Non-toxic and non-pathogenic properties, resistance to digestive juices and bile, nourishment of the digestive tract, antagonistic activity against pathogens, ability to survive in high populations, and the capacity to generate antimicrobial compounds are the criteria outlined by Gaggia *et al.* (2010) for safe probiotics. According to Peng *et al.* (2016), the use of *Lactobacillus plantarum* as a probiotic can increase the growth of broiler chickens by increasing the population of lactic acid bacteria in the cecum and ileum and reducing the content of *Escherichia coli* in the cecum.

As these bacteria utilize soybean SOS derived from soybean meal extract (SME) as a substrate, they are anticipated to increase lactic acid and short-chain fatty acid (SCFA) production, which decreases the pH of the small intestine. Consequently, the combination of *Lactobacillus plantarum* as a probiotic and SME as a prebiotic should result in a synergistic effect. This mechanism can trigger an increase in the LAB population to compete with pathogenic bacteria, namely *Escherichia coli*. There exists a negative correlation between the total LAB and the total *Escherichia coli* population. This means that an increase in total LAB is associated with a decrease in total Escherichia coli bacteria, while the opposite holds true. This occurrence is caused by competition for positions and nutrients in the diges-

tive tract. Conditions that inhibit the development of *Escherichia coli bacteria* have positively impacted broiler chickens' digestive tract and health. Several studies have found that combining prebiotics and probiotics as synbiotics in broiler feed can increase production performance. They combined prebiotics and probiotics as synbiotics increase body weight and feed conversion ratio (FCR) better when compared to a single use (Setyaningrum *et al*., 2019; Yuanita *et al*., 2019). In contrast to probiotics or prebiotics alone, Mohammed *et al.* (2018) and Abdel-Wareth *et al.* (2019) found that synbiotic supplementation enhanced weight gain in broiler chickens more effectively.

Body health is related to the health of the small intestine, which is positively correlated with the health of the digestive tract. A healthy digestive tract can optimize nutrient absorption (Qiu *et al*., 2023). The condition of a healthy digestive tract is expected to increase the digestibility of nutrients, especially crude protein, which affects protein utilization. It leads to productivity or body weight gain in livestock (Sholiha *et al.,* 2022). The health of broiler chickens is also related to the body's response to stress, which the antioxidant status can indicate. Stress on the normal functioning of the digestive organs can disrupt the balance between production and elimination of *reactive oxygen species* (ROS). High ROS levels in the intestine can destroy polyunsaturated fatty acids in cell membranes**,** which causes peroxide production and can lead to the production of *Malondialdehyde* (MDA) increase. This is consistent with the viewpoint expressed by Wadhwa *et al*. (2012) that ROS incite a radical peroxidation chain reaction by counteracting the double bonds present in unsaturated fatty acids with reactive hydrogen atoms. When ROS production in the body exceeds the ability of cells to detoxify ROS, oxidative stress will occur, characterized by lipid peroxidation, where when lipid peroxides are unstable, they decompose to form a series of compounds, including reactive carbonyl compounds. These polyunsaturated fatty acids are decomposed into malondialdehyde (MDA) and 4-hydroxyalkenals (HAE). Stress conditions can increase the number of peroxisomes, increasing the concentration of free radicals and cell oxidants produced by peroxisome oxidation (Całyniuk *et al.,* 2016). The results of free radicals can cause oxidative damage and lipid peroxidation in cell membrane components and produce the final product, namely MDA (Całyniuk *et al.,* 2016). Malondialdehyde is a byproduct of fat peroxidation, according to Deng *et al*. (2014), and it may indicate cellular injury. The higher the MDA value, the higher the level of oxidative stress (Boostani *et al*., 2015). *Superoxide dismutase* (SOD) is an antioxidant enzyme that is the first and primary line against oxidative damage (Kismiati *et al.,* 2021). The higher the SOD activity, the higher the antioxidant activity. The addition of synbiotics was able to increase SOD activity and decrease blood MDA levels, which is consistent with the results of Song *et al*. (2022).

Based on these synbiotic potentials, soybean meal extract combined with *Lactobacillus plantarum* is expected to become a synbiotic in improving the health, protein digestibility, and performance of broiler chickens. The study examined the effect of adding soybean meal extract (SME) and *Lactobacillus plantarum* on broiler chickens' health, protein digestibility, and performance.

MATERIALS AND METHODS

Animal and Experimental Design

The research was conducted in vivo for 35 days, from October to November 2022, in the Poultry Cage, teaching farm of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, Semarang. The material used was 192 broiler chickens with Cobb CP 707 *strain* from the production of PT Charoen Pokphand aged 8 days with a body weight of 137.89 ± 3.7 g. This study was conducted using a completely randomized design with 6 treatments and 4 replicates, with 8 replicates each. The treatments tested were T0: basal diet as control, T1: basal diet + *Lactobacillus plantarum* 1.2%, T2: basal diet +

Table 1. Composition of feed ingredients and nutritional content of rations

Feed Ingredients	Composition (%)
Corn	50.51
Pollard	16.74
Soybean meal	21.90
Meat bone meal	10.00
$CaCO_3$	0.30
Premixes	0.25
Lysine	0.10
Methionine	0.20
Nutrients	
Metabolic Energy (kcal/kg) *	3,034.58
Crude protein (%) **	21.44
Crude Fiber (%) **	4.42
Crude Fat (%) **	3.40
Calcium (%) **	1.44
Phosphorus (%) **	0.73
Methionine (%) ***	0.48
Lysine (%) ***	1.15
Arginine (%) ***	1.33

* Based on the Bolton Formula = 40.81 [0.87(CP+2.25×CF+BETN)+k] (Bolton, 1967)

** Result of Analysis of Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang (2022)

*** Amino acids were analyzed at Balai Mutu dan Sertifikasi Pakan, Bekasi, Indonesia (2022)

SME 0.15%, T3: basal diet + SME 0.30%, T4: basal diet + SME 0.15% + *Lactobacillus plantarum* 1.2%, T5: basal ration + SME 0.30% + *Lactobacillus plantarum* 1.2%.

Preparation of Soybean Meal Extract

Soybean meal was obtained at Kendal Market, Indonesia. Soybean meal extract was made by extraction based on the method of Krismiyanto *et al.* (2014). The extraction process was done by mixing distilled water on soybean meal (250 ml/200 g) and 96% ethanol (750 ml/200 g). The process began with soybean meal that has been in the form of flour mixed with distilled water and 96% ethanol, then heated in a water bath at 80°C. After boiling, the mixture was stirred for 30 minutes before being filtered to obtain the filtrate. The filter paper used was Whatman 41 paper with a diameter of 110 mm and a pore size of 20 µm. The filtration results were precipitated for 24 hours at room temperature before being separated with water. The precipitate obtained was then dried and milled into flour. *Lactobacillus plantarum* was obtained from the Microbiology Laboratory, Universitas Gadjah Mada, Yogyakarta, Indonesia. The composition of feed ingredients and the nutrient content of the rations are listed in Table 1. Feed in the form of the crumble was given ad libitum during the study (28 days).

Sampling and Analyses

Variables measured were broiler performance, lactic acid bacteria (LAB) population, *Escherichia coli population*, small intestine pH, protein consumption and digestibility, and antioxidant activity (MDA and SOD). Growth performance observed included total daily weight gain (DWG), total daily feed intake (DFI), feed conversion ratio (FCR). Daily weight gain was determined by comparing the subjects' weight at the start of treatment (8 days) and at the end of the study (35 days), with the difference between the two values calculated and divided by 28 days. The calculation of FCR with the results of calculating daily feed intake is divided by daily body

weight gain.

Total LAB and *Escherichia coli* were calculated using *the total plate count* (TPC) method. The formula used to measure total LAB and *Escherichia coli* was calculated based on Fardiaz's (1993) formula. Intestinal pH data collection was carried out with all contents of the small intestine homogenized, after which it was measured using a pH meter to determine the pH level in the small intestine.

Sampling for analysis of MDA and SOD were carried out on chickens aged 35 days, one for each replicate. The selection of broiler chickens to be sampled was based on the average body weight in all experimental units. All broiler chickens were weighed first, then the average was found and the broiler chickens in each treatment and replication were selected that were close to the average. Samples were taken by taking blood from the wing vein as much as 3 ml and then collected in a test tube containing EDTA. The sample was centrifuged at 3,500 rpm for 10 minutes to separate plasma and serum. MDA activity was measured by the reactive substances assay method for thiobarbituric acid (TBA) based on the method of Agusetyaningsih *et al.* (2022). MDA concentration is expressed in units of nmol/ml. SOD activity was tested according to the ability of the samples with the aim of suppressing the auto-oxidation of pyrogallol. The mixture to be tested consisted of 50 mM Tris-HCl (pH 8.2), 1 mM pentaacetic acid diethylenetriamine, and a sample. The reaction began with the addition of pyrogallol with a final concentration of 0.2 mM and then the absorption value was calculated kinetically. The SOD concentration is in units of U/ml (Agusetyaningsih *et al*., 2022).

Protein digestibility data was collected using the total collection method by adding 0.5% Fe_2O_3 indicator from the ration. Chickens were kept in 24 battery cages containing one chicken in each experimental unit. On the first day, the chickens were given treatment rations containing additional indicators, and their behavior was monitored for 24 hours. In addition, a tray was placed beneath the battery cage to collect excreta. The Excreta collection started when the red excreta first appeared and continued until the second day after the excreta changed color. In the subsequent stage, on the second day, broiler chickens were provided with treatment rations without indicators. On the third day, indicators were reintroduced, and on the fourth day, the rations were given without indicators again. The tray, serving as a container for excreta, was replaced daily. Excreta was collected daily and sprayed with 0.2 N HCL every 2 hours at each reservoir (Krismiyanto *et al*., 2022). The collected excreta were dried and ground, then analyzed for crude protein content using the Kjeldahl method. Excreta protein content was analyzed at the Laboratory of Nutrition and Feed Science, Faculty of Animal and Agricultural Sciences, Universitas Diponegoro, to calculate protein digestibility. Calculation of protein digestibility based on the following formula according to McDonald *et al.* (1997)/

Statistical Analysis

The data were analyzed using ANOVA (significance level at 5%), continued with Duncan's multiple range test to determine the differences among treatments.

RESULTS AND DISCUSSION

Performance of Broiler Chickens

The effect of adding soybean meal extract and *Lactobacillus plantarum* on broiler performance is presented in Table 2. Treatment had a significant effect ($p<0.05$) on daily body weight gain in the 2nd and 4th weeks, as well as total daily body weight gain (28 days). Beginning with the 3rd to the 4th week, the treatment significantly changed daily feed intake parameters and total daily feed intake (28 days). In contrast, the treatment had a significant effect on FCR parameters in the 2nd week and total FCR (28 days).

Total daily weight gain (28 days) at T5 was significantly higher than at T3, T2, T1, and T0, but not different from T4. Furthermore, in the 2nd week, the daily body weight gain of T5 was significantly different from T3, T2. T1 and T0, but

Table 2. Broiler performance

Variables	T0	T1	T2	T3	T4	T5	SEM	p-value
DWG(g)								
Week 1 (7-14)	26.85	27.80	26.55	28.23	27.36	28.65	0.38	0.64
Week 2 (14-21)	46.01^{c}	49.48^{bc}	51.84^{b}	53.93^{b}	58.83^{a}	59.92^{a}	1.19	<0.001
Week 3 (21-28)	41.72	42.43	46.23	51.72	50.34	45.84	1.37	0.19
Week 4 (28 – 35)	67.67^{bc}	66.86^{bc}	63.29^{c}	71.19^{bc}	72.69^{b}	81.93^{a}	1.61	<0.001
DWG total (28 days)	45.56^{c}	46.64^{c}	46.98^{c}	51.27^{b}	52.30^{ab}	54.09^{a}	0.73	<0.001
DFI (g)								
Week 1 (7-14)	54.38	54.39	53.42	57.54	55.07	55.78	0.71	0.4706
Week 2 (14-21)	88.84	81.53	77.70	89.36	90.94	84.21	1.54	0.0716
Week 3 (21-28)	95.46^{b}	97.03^{b}	107.34^{a}	116.03^{a}	108.60^{a}	109.62^{a}	1.91	0.0019
Week 4 (28 – 35)	107.84^{c}	110.95^{bc}	108.99^{bc}	115.66^{abc}	119.69^{ab}	122.54^{a}	1.73	0.05
DFI total (28 days)	74.89^{b}	74.17^{b}	74.52^{b}	81.13^{a}	80.27^{a}	79.94^{a}	0.79	0.002
FCR								
Week 1 (7-14)	2.03	1.96	1.98	2.04	2.02	1.96	0.02	0.87
Week 2 (14-21)	1.94^{a}	1.65^{b}	1.50^{bc}	1.66^{b}	1.55^{bc}	1.41^{c}	0.04	<0.001
Week 3 (21-28)	2.29	2.30	2.32	2.35	2.17	2.41	0.06	0.94
Week 4 (28 – 35)	1.60	1.66	1.72	1.65	1.66	1.50	0.03	0.55
FCR total (28 days)	1.64^{a}	1.60^{ab}	1.59^{ab}	1.58^{ab}	1.54^{bc}	1.48^{c}	0.01	0.011

^{ab} Means different superscripts in the same row significantly differ (P<0.05). T0: basal diet as control, T1: basal diet + *Lactobacillus plantarum* 1.2%, T2: basal diet + SME 0.15%, T3: basal diet + SME 0.30%, T4: basal diet + SME 0.15% + *Lactobacillus plantarum* 1.2%, T5: basal ration + SME 0.30% + *Lactobacillus plantarum* 1.2%. DWG: Daily Weight Gain. DF1: Daily Feed Intake. FCR: feed conversion ratio. SEM: standard error of the mean

have the same value as T4. Meanwhile, T5 had a significantly larger daily body weight gain than the others in the 4th week. Total Daily Feed Intake at T5 was significantly higher than those at T2, T1, and T0 but not different from T4 and T3. Furthermore, in the 3rd week, the daily feed intake of T5 was significantly higher than T1 and T0, but had the same value as T4, T3 and T2. Meanwhile, on the 4th week, daily feed intake of T5 was significantly higher than T2, T1 and T0, but had the same value as T4 and T3. Total feed conversion was significantly lower in the T5 treatment than in T3, T2, T1, and T0, but not different from T4. FCR in the 2nd week showed that T5 results were significantly lower than T0, T1 and T3, but had the same value as T4 and T2.

The results showed that the addition of soybean meal extract and *Lactobacillus plantarum* was able to improve the performance of broiler chickens. Increasing SME's addition level increases total daily weight gain, total daily feed intake, and lower total feed conversion values. Several studies have found that adding synbiotics to broiler chickens can increase weight gain and feed efficiency (see Abdel-Hafeez *et al.,* 2017). The synergistic efficacy of the synbiotic combination of soybean meal extract and *Lactobacillus plantarum* is supported by the collaborative action of soybean oligosaccharides within the soybean meal extract and the probiotic activity of *Lactobacillus plantarum*. In this synergy, the soybean meal extract acts as a substrate for the probiotics, allowing them to produce short-chain fatty acids (SCFA) and lactic acid, which contribute to an acidic environment in the digestive tract. This atmosphere promotes the growth of lactic acid bacteria while suppressing the population of *Escherichia coli*. Sapsuha *et al.* (2023) suggested that these settings can preserve microbial balance, improve digestive function, and boost nutrient absorption in the small intestine of broiler chickens. The results showed that the feed utilization was higher than the control treatment (T0). Nutmeg pulp extract can promote the development of *Lactobacillus plantarum* bacteria, and its incorporation into broiler chickens as a synbiotic has been demonstrated in research by Sapsuha *et al*. (2023). Riad *et al*. (2010) additionally document that feed conversion can be diminished through the use of prebiotics, probiotics, and their combinations in the diet of broiler chickens. The decrease in feed conversion can

be attributed to the alteration of intestinal pH caused by the combination of the two substances. This, in turn, influences the composition of microorganisms and nutrient assimilation, ultimately improving feed utilization efficiency. In order to increase the surface area for increased nutrition absorption, Awad *et al.* (2009) asserted that additives have the capacity to raise villi height and intestinal length. This improved nutritional absorption has a direct impact on increasing the body weight of broiler chickens. Mousavi *et al.* (2015) suggested that synbiotics can increase body weight gain in broiler chickens. In line with report of Bogucka *et al.* (2019), synbiotic supplementation increases body weight gain in the first maintenance period.

Bacterial Population and pH in the Small Intestine

The effect of adding soybean meal extract and *Lactobacillus plantarum* on the bacterial population and pH in the small intestine is presented in Table 3. The treatment significantly affected ($p<0.05$) the population of lactic acid bacteria, *Escherichia coli*, and intestinal pH. The LAB population at T5 was significantly higher than T2, T1, and T0, but had the same value as T4 and T3. The *Escherichia coli* population at T5 was significantly lower than T1 and T0, but not different from T4 and T3. Intestinal pH at T5 was significantly lower than T0, T1, T2, and T3, but not different from T4.

The results indicated that the addition of *Lactobacillus plantarum* and soybean meal extract can improve the condition of the microflora in the small intestine. With increasing concentrations of soybean meal extract, the population of lactic acid bacteria increased and the population of Escherichia coli decreases. Moreover, in line with the pH of the small intestine, when the level of SME addition increased, the pH of the small intestine decreased. Soybean meal extract contains soybean oligosaccharides as prebiotics and *Lactobacillus plantarum* as probiotics capable of working in synergy to become synbiotics. In contrast, soybean oligosaccharides in SME can be used as substrates for *Lactobacillus plantarum*. In the process, it can increase the production of SCFA and lactic acid. Prancute *et al.* (2014) asserted that plant-derived non-digestible oligosaccharides can increase lactic acid bacteria's growth and activity while enhancing the generation of volatile fatty acids. The increase in SCFA and lactic acid impact decreases the pH in the digestive tract. Krismaputri *et al.* (2016[b]) showed that the mechanism of decreasing pH occurs because organic acids SCFA can dissociate (split or separate) inside cells to produce positive hydrogen ions (H^+), which results in a decrease in cell pH and an acidic environment in the digestive system. The research results by Sunu *et al*. (2021) showed that synbiotics can significantly lower the pH of the small intestine in the duodenum, jejunum, and ileum. An acidic pH atmosphere in the digestive tract is a good condition because it triggers the growth of LAB. Low pH in the digestive tract can increase LAB's growth and inhibit pathogenic bacteria's growth. Hidayat *et al*. (2018) asserted that the ability of LAB to persist at low pH is due to the fact that the intracellular pH can adapt to a decrease in extracellular pH, preventing the formation of a significant proton gradient.

Malago and Koninkx (2011) indicated, an

Table 3. Bacterial population and pH in the small intestine

Variables	T0	T1	T2	T3	T4	T5	SEM	p-value
LAB (log cfu/g)	6.99[c]	8.99[b]	8.98[b]	9.30[ab]	9.31[ab]	10.26[a]	0.23	<0.001
E. coli (log cfu/g)	4.54[a]	2.40[b]	2.15[bc]	2.15[bc]	1.75[bc]	1.50[c]	0.24	<0.001
Intestinal pH	6.40[a]	6.28[ab]	6.17[bc]	6.05[c]	5.91[d]	5.90[d]	0.04	<0.001

[ab] Means different superscripts in the same row significantly differ ($P<0.05$). T0: basal diet as control, T1: basal diet + *Lactobacillus plantarum* 1.2%, T2: basal diet + SME 0.15%, T3: basal diet + SME 0.30%, T4: basal diet + SME 0.15% + *Lactobacillus plantarum* 1.2%, T5: basal ration + SME 0.30% + *Lactobacillus plantarum* 1.2%. LAB: Lactic acid bacteria. SEM: standard error of the mean

increased LAB population can prevent harmful bacterial growth by competing for resources in the gut or binding sites on the intestinal epithelium. Most intestinal pathogens must attach to the intestinal epithelium to colonize the intestine and cause disease. Yang *et al.* (2015) stated that increasing the production of intestinal mucus, which functions as a phytochemical barrier to protect epithelial cells, is another mechanism for inhibiting microorganisms in the intestine. Mack *et al*. (1999) and Mack et al. (1999) reported that *Lactobacillus plantarum* is shown to increase the expression of MUC2 and MUC3 mRNA in intestinal cells and inhibit the adhesion of enteropathogenic *E. coli*. Mookiah *et al.* (2014) further stated that the administration of synbiotics to broiler chickens influences the composition of beneficial microflora in the cecum, specifically resulting in a reduction of *E. coli* and an increase in populations of lactobacilli and bifidobacteria. The results of Dibaji *et al.* (2014) showed that adding synbiotics lowered the *Escherichia coli* population as well as the total coliform population in broiler chickens' intestines. Numerous studies concur with Neeraj (2016) that synbiotics can increase the population of lactic acid bacteria in the duodenum, jejunum, and ileum while decreasing the population of *Escherichia coli* in the ileum.

Protein Consumption and Digestibility

The effect of adding soybean meal extract and *Lactobacillus plantarum* on protein consumption and digestibility is presented in Table 4. Protein digestion and protein consumption were both significantly impacted by the treatment. Protein digestibility and protein consumption were both lowest at T5 compared to T4 and T3, but highest at T5.

The results showed that broiler protein digestibility can be improved by adding soybean meal extract and *Lactobacillus plantarum*. Protein digestibility was significantly impacted by treatment, while protein intake did not show a significant difference. The addition of SME, which includes *Lactobacillus plantarum* as a probiotic and soybean oligosaccharides (SOS) as prebiotics, has been shown to be highly effective. The amount of SME consumed was related to the amount of SOS that enters the body. These results were consistent with the rise in LAB populations and the decline in *Escherichia coli* and other pathogenic bacteria. By influencing the digestibility of nutrients and proteins, optimal intestinal microbial balance can enhance gut health. Pourabedin and Zhao (2015) stated, preventing the growth of harmful bacteria can enhance digestive tract health, resulting in optimal nutrient absorption. Sjofjan *et al.* (2021) reported that LAB can create protease enzymes that are useful for protein digestion in broiler chickens. Yang *et al.* (2015) mentioned that Lactic Acid Bacteria (LAB) produces bacteriocins, which are peptides with bactericidal activity. These bacteriocins play a role in combating pathogenic bacteria, inhibiting the growth, and preventing the attachment of harmful bacteria. This activity contributes to the enhancement of intestinal health. The research results of Mora *et al*. (2019) showed that broiler chickens added with synbiotics in the ration have healthier mucosa than the control treatment.

Antioxidant Activity

The effect of adding soybean meal extract and *Lactobacillus plantarum* on antioxidant activity is presented in Table 5. The treatment had a significant effect on *malondialdehyde* and *superoxide dismutase*. The *malondialdehyde* level at T5 was significantly lower than at T2, T1, and T0, but not different from T4 and T3. Superoxide dismutase level in T5 was significantly higher than in the other treatments.

The results showed that supplementing chickens with soybean meal extract and *Lactobacillus plantarum* can increase antioxidant activity. In comparison to other treatments, T5 had the greatest SOD level. Mounir *et al*. (2022) asserted that the antioxidant mechanism of action may be associated with two primary categories and mechanisms of synbiotics. The first is complementary synbiotics, in which probiotics and prebiotics function as antioxidants on the host while acting independently. The second category

Table 4. Protein Consumption and Digestibility

Variables	T0	T1	T2	T3	T4	T5	SEM	p-value
Protein Consumption (g)	16.00[b]	15.90[b]	15.98[b]	17.39[a]	17.21[a]	17.14[a]	0.17	0.001
Protein Digestibility (%)	80.37[d]	82.92[c]	85.42[b]	86.69[ab]	87.99[a]	88.53[a]	0.66	<0.001

[ab] Means different superscripts in the same row significantly differ (P<0.05). T0: basal diet as control, T1: basal diet + *Lactobacillus plantarum* 1.2%, T2: basal diet + SME 0.15%, T3: basal diet + SME 0.30%, T4: basal diet + SME 0.15% + *Lactobacillus plantarum* 1.2%, T5: basal ration + SME 0.30% + *Lactobacillus plantarum* 1.2%. SEM: standard error of the mean

Table 5. Antioxidant Activity

Variables	T0	T1	T2	T3	T4	T5	SEM	p-value
Malondialdehyde (nmol/ml)	1.27[a]	1.03[b]	1.01[b]	0.89[bc]	0.87[bc]	0.80[c]	0.04	<0,001
Superoxide dismutase (U/ml)	2.55[e]	13.26[d]	16.33[c]	16.84[c]	18.88[b]	21.43[a]	1.28	<0,001

[ab] Means different superscripts in the same row significantly differ (P<0.05). T0: basal diet as control, T1: basal diet + *Lactobacillus plantarum* 1.2%, T2: basal diet + SME 0.15%, T3: basal diet + SME 0.30%, T4: basal diet + SME 0.15% + *Lactobacillus plantarum* 1.2%, T5: basal ration + SME 0.30% + *Lactobacillus plantarum* 1.2%. SEM: standard error of the mean

consists of synergistic synbiotics, in which prebiotics, despite potentially lacking antioxidant properties themselves, bolster and advance the antioxidant functionality of probiotics, thereby augmenting the collective antioxidant capacity of each constituent. Consistent with the viewpoint expressed by Choudhary *et al*. (2019), the antioxidant properties of a composition can be enhanced by combining probiotics with non-antioxidant oligosaccharide-based prebiotics.

Mounir *et al*. (2022) further stated that the mechanism of action of antioxidants is indeed influenced by the effects of probiotics or prebiotics alone, and particularly by their interaction in the form of synbiotics. Each component plays an important role in neutralizing free radicals. Cruz *et al*. (2021) asserted that probiotics have the capacity to impact the redox status of the host by means of various mechanisms: chelating metal ions, stimulating the host's antioxidant system via its antioxidant enzyme system, generating metabolites including butyrate and glutathione (GSH), which possess antioxidant activity, mediating antioxidant signaling pathways, regulating enzymes responsible for reactive oxygen species production, and modulating gut microbiota. Probiotics have also been reported to be able to indirectly control host cell oxidative stress by increasing ant oxidase activity, reducing ROS-producing enzymes, and regulating antioxidant signaling pathways (Yang *et al*., 2022; Zhang *et al.,* 2020). Gao *et al*. (2021) reported, *L. plantarum Y44* can lower oxidative stress by changing the makeup of the gut microbiota. Mounir *et al.* (2022) added that this strain results in changes in microbiota composition, glycerophospholipid levels, and oxidative stress markers, and that it can assist defend the host's antioxidant system by creating and releasing antioxidant metabolites. Meanwhile, Cheong *et al*. (2018) asserted, the role of prebiotics is that oligosaccharides can scavenge various radicals, such as DPPH and ABTS radicals. In oligosaccharides, the hydroxyl groups at locations C-2 and C-6 participate in the H atom transfer process with these radicals. When the combined effects of probiotics and prebiotics are considered, whether through complementary or synergistic pathways, there is

growing evidence that synbiotics have antioxidant activity. Report of Kleniewska *et al*. (2016) indicated, the synbiotic combination of L. casei and inulin protects the organism effectively against free radical damage.

MDA levels are metabolites produced by lipid peroxidation that can be used as an example of free radical activity in cells and hence as an indicator of oxidative stress. Boostani *et al.* (2015) stated that oxidative stress increases in direct proportion to the MDA value. Chen *et al*. (2018) reported that synbiotic supplementation (mixed with xylo-oligosaccharide, *Clostridium butyricum*, and *Bacillus subtilis*) can minimize malondialdehyde ileum buildup in broiler chicken at 42 days of age. SOD is an antioxidant enzyme that is the first and main line against oxidative damage (Kismiati *et al.,* 2021). *Superoxide dismutase*, which is produced by the liver, is vital in maintaining free radical levels in ROS form in normal settings, preventing an oxidation reaction that might harm the body (Ancuelo *et al*., 2021). Habashy *et al.* (2019) stated that the SOD mechanism converts oxidative peroxide (H_2O_2) into H_2O and O_2 in order to protect cells from the oxidative damage caused by H_2O_2. The higher the SOD activity, the higher the antioxidant activity. Mounir *et al.* (2022) reported, synbiotic supplementation can lower MDA levels, a lipid peroxidation marker, and enhance GSH levels, nitric oxide, and total antioxidant capacity, a measure of the quantity of scavenged free radicals. In order to prevent oxidative stress, Na and Surh (2008) stated that synbiotic supplementation is crucial as a detoxification agent for free radical production. Popović *et al*. (2015) further asserted that the administration of synbiotic supplementation to broiler chickens enhances their antioxidant activity and mitigates the detrimental effects of free radicals. The findings presented in this study are consistent with the results reported by Song *et al*. (2022), which demonstrate that the inclusion of synbiotics can enhance SOD activity and decrease blood MDA levels. The durability of broiler chickens can be increased through synbiotic supplementation, as demonstrated by an increase in SOD score and a decrease in MDA score (Kismiati *et al.*, 2021).

CONCLUSION

This study reveals that supplementing soybean meal extract with 0.30% and *Lactobacillus plantarum* with 1.2% (T5) is effective in increasing protein digestibility and optimizing performance in broiler chickens.

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