



Hematological and Performance Variables of Male Broiler Chickens Fed with *Moringa oleifera* Extract and Probiotic in Drinking Water

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ABSTRACT

Moringa oleifera is a potential plant that can be used to improve immunity, the gut health of broiler chickens, and reduce the number of pathogens in the intestine. Probiotics are non-pathogenic microbes that can balance the microflora in the digestive tract and improve poultry production performance. The objective of this study was to demonstrate the impact of adding probiotic *Lactobacillus* sp. and *M. oleifera* extract to broiler feed. In this study, a factorial, completely randomized design was used. This research used 900 animals, which were divided into two factors: the first factor was the dose of *M. oleifera* (0%, 1%, and 2%) and the second factor was the dose of probiotics (0%, 1%, and 2%). The treatment was 9 (3 x 3 factorial design), with each consisting of 10 replications and each replication consisting of 10 chickens. *M. oleifera* extract and probiotics were supplemented in drinking water during the 5-week experimental period. *Ad libitum* supplies of food and drink were provided. The results revealed that there was an interaction ($p < 0.05$) between the doses of *M. oleifera* extract and probiotic on leucocytes, monocytes, granulocytes, thrombocytes, and hematocrit when *M. oleifera* extract and probiotics were added. There was no interaction between the doses of *M. oleifera* extract and probiotics on the levels of lymphocytes, hemoglobin, and erythrocytes, but all blood profile values were within the normal range. The feed intake, feed conversion ratio, and daily body weight gain showed significant differences ($p < 0.05$) that increased between treatments. It could be concluded that using *M. oleifera* extract and *Lactobacillus* sp probiotics as feed additives did not alter the normal blood profile values and could increase the performance of male broiler chickens and income over feed cost (IOFC).

Keywords: blood profile; growth performance; health; *Lactobacillus* sp; *Moringa oleifera* extract

INTRODUCTION

Probiotics are non-pathogenic bacteria that can be utilized to boost the balance of microbes in the stomach, thus enhancing host health, growth performance, feed efficiency, and livestock productivity (Khanian *et al.*, 2019; Lokapirnasari *et al.*, 2022a; Yulianto & Lokapirnasari, 2018). Sugiharto (2016) showed that the species of microorganisms currently used in probiotic preparations are varied, including *Lactobacillus*

plantarum, *L. acidophilus*, and *L. casei*. The benefits of probiotics include enhancing the immune system, protecting the gastrointestinal tract from infectious agents (Scalfaro *et al.*, 2017; Qin *et al.*, 2018; Yulianto *et al.*, 2021), reducing blood cholesterol levels (Dixon *et al.*, 2020), and enhancing the body's ability to absorb nutrients (Jäger *et al.*, 2018; Moghaddam *et al.*, 2020). These benefits could increase performance and poultry production (Lokapirnasari *et al.*, 2019; Agustono *et al.*, 2022; Karwanti *et al.*, 2023; Lokapirnasari *et al.*,

2023). Probiotic supplementation caused a statistically significant increase in the erythrocyte count, hemoglobin concentration, and hematocrit values of chickens (Alkhalif *et al.*, 2010).

In general, Lactobacilli, Bifidobacteria, *Enterococcus faecium*, *Enterococcus faecalis*, and Saccharomyces are the probiotic types acknowledged as safe for broiler and human consumption (generally recognized as safe) (Zawistowska-Rojek & Tyski, 2018; Sugiharto, 2016). Several probiotics that affect broiler growth performance include the probiotic *Lactobacillus fermentum*, *Lactobacillus plantarum* (Lokapirnasari *et al.*, 2020), *Lactobacillus casei*, and *Lactobacillus rhamnosus* (Andriani *et al.*, 2020). The benefits of using probiotics include the increased bird survival, improved immunological response, decreased gastrointestinal upsets, increased growth rates, and improved feeding efficiency (Al-Aqaby *et al.*, 2021). Probiotics may provide health benefits such as improved digestion, activation of gastrointestinal immunity, and higher natural resistance to enteric diseases. The blood profile of livestock is an indicator that can be used to identify the health status of animals. The blood profile includes leukocytes, erythrocytes, lymphocytes, monocytes, granulocytes, platelets, hematocrit, and hemoglobin (Hidayat *et al.*, 2020). Therefore, prebiotics can be added to foods to promote the growth of probiotics.

Moringa oleifera is used as a traditional medicinal plant in many tropical and subtropical countries for pharmaceutical or nutraceutical development (Islam *et al.*, 2021). Flavonoids, steroids, tannins, saponins, phlorotannin, and terpenoids are among the functional substances or metabolite products found in leaf preparations of *M. oleifera* (Kashyap *et al.*, 2022). *M. oleifera* also contains nutrients, vitamins, and minerals (Islam *et al.*, 2021). Carotene levels are considerable in dried leaves, ranging from 23.33 to 39.6 mg/100 g of dry weight. *M. oleifera* contains protein (29.22%), lipids (5.70%), carbohydrates (45.69%), oligosaccharides, and non-digestible dietary fiber (Caicedo-Lopez *et al.*, 2019).

The bioactive content of *M. oleifera* includes flavonoids such as quercetin and kaempferol, which function as antioxidants; phenolic acids, phytosterols, alkaloids, sugars, organic acids (Coppin *et al.*, 2013); and several minerals such as iron, calcium, magnesium, potassium, copper, and zinc (Kasolo *et al.*, 2010; Gopalakrishnan *et al.*, 2016). Beta-carotene, vitamin B (pyridoxine, nicotinic acid, and folic acid), vitamins C, D, and E, as well as numerous critical amino acids, including leucine, isoleucine, phenylalanine, and tryptophan, are also present in *M. oleifera* (Ramadurai *et al.*, 2010; Mbikay, 2012; Rodríguez-Pérez *et al.*, 2015). Dry leaves of *M. oleifera* extracted using 70% ethanol contain high crypto-chlorogenic acid and iso-quercetin (every 100 g of extract contains 13.23 g of chlorogenic acid equivalents and a high total of flavonoids, i.e. every 100 g of extract contains 6.20 g of iso-quercetin equivalents) (Laili *et al.*, 2019). In this study, the combination of *M. oleifera* and probiotic was used because the *in vitro* evaluation of *M. oleifera* extract could increase the growth of the bacteria probiotic ($p < 0.05$). The study showed that *M. oleifera* functions as a prebiotic

because it can increase the growth of probiotics *in vitro* (Karwanti *et al.*, 2023).

However, the study combining *Lactobacillus sp.* probiotics and *M. oleifera* extract as prebiotics has several limitations. These two ingredients can be combined because probiotics and *M. oleifera* extract can improve chicken performance and blood profiles. Thus, the aim of this study was to prove the effectiveness of the combination of *M. oleifera* extract and *Lactobacillus sp.* probiotics on blood profiles (e.g., leucocyte, monocytes, granulocytes, erythrocyte, lymphocytes, thrombocyte hemoglobin, hematocrit hematocrit), growth performance (daily body weight gain), feed intake, feed conversion ratio of broilers chicken and income over feed cost (IOFC).

MATERIALS AND METHODS

Ethical Clearance

Ethical clearance of the study was approved by the Animal Care and Use Committee of Universitas Brawijaya (No.029-KEP-UB-2021).

Experimental Design

This research was conducted from December 2021 to June 2022. This research used 900 animals, which were divided into two factors: the first factor was the dose of *M. oleifera* (0%, 1%, and 2%) and the second factor was the dose of probiotics (0%, 1%, and 2%). The interaction between the two was performed as a 3 × 3 factorial experiment, with 10 replications, each replication consisting of 10 chickens with a completely factorial randomized design. For hematological and performance sampling, samples were obtained from 90 treated chickens.

In this study, a factorial, completely randomized design was used. The first factor was *M. oleifera* extract and the second factor was the probiotic *Lactobacillus sp.*, each with doses 0 %, 1%, and 2% (w/v) concentration of 1.2×10^9 cfu/mL). Throughout the 35-day testing period, probiotics and an extract of *M. oleifera* were added to the drinking water. *Ad libitum* supplies of feed and drink were provided.

The feed was given twice a day, as much as 150 g/chickens/day. Broiler chickens were fed a commercial broiler diet with the following specifications: dry matter, 91.97%; ash, 9.28%; crude protein, 20.71%; ether extract, 6.36%; crude fiber, 7.43%; nitrogen-free extract, 48.18%; and metabolizable energy (ME): 2938.60 kcal/kg. Drinking water was provided *ad libitum* during the treatment.

Isolate Preparation

For preparation and cultivation of the probiotic, *Lactobacillus sp.* was separately cultured in de Man–Rogosa– Sharpe (MRS broth) (Oxoid, Thermo Fisher Scientific Inc) for 24 h at 37 °C (Rahmati, 2017). *Lactobacillus sp.* used in this research is Gram positive, negative on the catalase test, non-motile, and rod-

shaped. In a previous *in vitro* study, this *Lactobacillus* sp. showed the ability to act as a probiotic because that isolate was acid tolerant and bile acid tolerant and produced bacteriocin, which was able to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. The dose of 1% and 2% probiotic culture was carried out by taking 1 mL and 2 mL of isolate dissolved in 100 mL of drinking water, respectively. The probiotic added to drinking water was adjusted to a concentration of 1.2×10^9 cfu/mL.

Extraction of *M. oleifera*

The extraction procedure was conducted based on a modification of research by Charde *et al.* (2011). *M. oleifera* leaf powder weighing 2 kg was macerated in 2 L of 70% ethanol three times daily at room temperature (24-25 °C) before being filtered through Whatman paper. *M. oleifera* macerate was extracted using a renewed solvent multiple times, and the solvent was eventually evaporated from the mixture using rotary evaporation (60 °C, 50 rpm). *M. oleifera* extract with 1% and 2% doses was prepared by weighing 1 g and 2 g of *M. oleifera* extract and dissolved in 100 mL distilled water.

Sample Collection

Blood sampling data were collected from 35-day-old broilers. Blood samples were taken from the brachial vein using 23G \times $\frac{3}{4}$ gauge needles once the trial period was over. At least 1 mL of blood was drawn and placed in sterile EDTA tubes with anticoagulants. To prevent the lysis of blood clots, the tubes are shaken slowly and stored at low temperatures (18 °C). A cooler box is used to store blood that will be examined in the laboratory. Blood profiles were examined according to the protocol (Charde *et al.*, 2011).

Feed intake, feed conversion ratio, and body weight gain variables were calculated as follows (Karwanti *et al.*, 2023; Lokapirnasari *et al.*, 2023; Lokapirnasari *et al.*, 2022b; Hadieva *et al.*, 2021): The feeds offered and unconsumed feed were weighed and recorded to determine the amount of feed intake. Feed intake was calculated by reducing the feed offered with the remaining feed (unconsumed feed) using the following equation: Feed intake (g) = Feed offered (g) - remaining feed (g). Feed intake (FI) was evaluated weekly and subsequently re-estimated for a single bird. Feed conversion ratio (FCR) was obtained on the 35th day of age. The FCR was calculated by dividing the amount of feed intake by the body weight gain in that week using the following equation: FCR = feed intake/body weight gain. Average daily weight gain (ADWG) was calculated at 35 days of age.

Statistical Analysis

The data collected during this study were statistically analyzed using a completely randomized factorial design. All data were tested for normality of distribution and homogeneity of variance. Differences between means were detected using two-way analysis of

variance. Differences between means were determined using Duncan's test ($p < 0.05$).

RESULTS

The average number of hematological parameters for treating *M. oleifera* extract and probiotic *Lactobacillus* sp. in broiler chickens are listed in Table 1. The results showed that there was an interaction ($p < 0.05$) between the use of *M. oleifera* extract and the probiotic *Lactobacillus* sp. on leucocyte levels during the 5 weeks of the administration period. The lowest leucocyte values were found in treatment groups (a0b2), (a1b2), (a2b0), (a2b1), (a1b1), and (a0b1), which did not differ from treatment (a2b2). High leukocyte values were found in the treatment (a0b0).

Erythrocyte levels showed no interaction ($p > 0.05$) between the use of *M. oleifera* extract and the probiotic *Lactobacillus* sp. on the erythrocyte levels in the 5 weeks of the administration period. The results showed that there was no significant difference between the use of *M. oleifera* extract and the probiotic *Lactobacillus* sp. on erythrocyte levels.

The lymphocyte level result showed no interaction between the use of *M. oleifera* extract and the probiotic *Lactobacillus* sp. on lymphocytes in the 5 weeks of the administration period. The results showed that there was no significant difference between the use of *M. oleifera* extract and the probiotic *Lactobacillus* sp. on lymphocyte levels.

Monocyte levels showed that there was an interaction ($p < 0.05$) between the use of *M. oleifera* extract and the probiotic *Lactobacillus* sp. on monocytes in the 5 weeks of the administration period. The lowest monocyte value was found in treatment (a1b1), which was consistent with treatments (a0b2), (a1b0), (a1b2), (a2b0), (a2b1), and (a2b2). A high monocyte value was found in treatment (a0b0), which is consistent with treatments (a0b1) and (a0b2).

The granulocyte levels showed an interaction ($p < 0.05$) between the use of *M. oleifera* extract and the probiotic *Lactobacillus* sp. on granulocytes in the 5 weeks of the administration period. The lowest granulocyte level was found in treatment (a0b0), which was not different from treatment (a0b1). High granulocyte levels were found in treatment (a2b1), which was not different from treatments (a2b0), (a2b0), (a2b2), (a1b0), (a1b1), (a1b2), and (a0b2).

The result of thrombocyte levels showed an interaction ($p < 0.05$) in the 5 weeks of the administration period. The highest thrombocyte value is present for treating a0b0, which differs from all treatments, whereas a low thrombocyte value is in all treatments except a0b0.

The result of hematocrit levels showed an interaction ($p < 0.05$) in the 5 weeks of the administration period. The lowest hematocrit values were found for treating a1b1, consistent with the treatments of a2b1, a2b0, a1b2, a2b2, a0b1, a0b2, and a0b2. The highest hematocrit values were found for treating a1b0 and a0b0, which were not significantly different from the treatments of a2b1, a2b0, a1b2, a2b2, a0b1, a0b2, and a0b2.

Table 1. Average number of hematological parameters for treating *Moringa oleifera* extract and probiotic *Lactobacillus* sp. in broiler chickens

Variables	Probiotic <i>Lactobacillus</i> sp.	<i>Moringa oleifera</i> extract		
		a0 (0%)	a1 (1%)	a2 (2%)
Leucocytes (10 ³ /mm ³)	b0 (0%)	27.60 ± 1.10 ^c	23.20 ± 0.70 ^b	21.45 ± 1.15 ^a
	b1 (1%)	21.65 ± 0.15 ^a	21.50 ± 0.30 ^a	21.50 ± 1.40 ^a
	b2 (2%)	20.90 ± 0.00 ^a	21.05 ± 0.45 ^a	21.90 ± 0.90 ^{ab}
Erythrocytes (10 ⁶ /mm ³)	b0 (0%)	2.60 ± 0.00	2.65 ± 0.05	2.50 ± 0.30
	b1 (1%)	2.55 ± 0.15	2.55 ± 0.05	2.60 ± 0.20
	b2 (2%)	2.70 ± 0.10	2.70 ± 0.10	2.60 ± 0.10
Lymphocytes (%)	b0 (0%)	7.00 ± 1.00	6.50 ± 0.50	7.50 ± 0.50
	b1 (1%)	6.50 ± 1.50	6.50 ± 1.50	5.50 ± 1.50
	b2 (2%)	6.00 ± 2.00	6.50 ± 0.50	7.00 ± 0.00
Monocytes (%)	b0 (0%)	9.00 ± 0.00 ^c	7.00 ± 1.00 ^{ab}	6.50 ± 0.50 ^{ab}
	b1 (1%)	8.00 ± 0.00 ^{bc}	6.00 ± 1.00 ^a	6.50 ± 1.50 ^{ab}
	b2 (2%)	7.50 ± 1.50 ^{abc}	6.50 ± 0.50 ^{ab}	7.00 ± 1.00 ^{ab}
Granulocytes (%)	b0 (0%)	84.00 ± 1.00 ^a	86.50 ± 0.50 ^{ab}	86.00 ± 1.00 ^{ab}
	b1 (1%)	84.00 ± 1.00 ^a	86.50 ± 0.50 ^{bc}	86.00 ± 1.00 ^{bc}
	b2 (2%)	85.50 ± 1.50 ^{ab}	87.00 ± 0.00 ^{bc}	87.50 ± 0.50 ^c
Thrombocyte (10 ³ /mm ³)	b0 (0%)	295.00 ± 3.00 ^a	249.00 ± 16.00 ^b	239.50 ± 8.50 ^b
	b1 (1%)	235.50 ± 15.50 ^b	239.50 ± 18.50 ^b	240.50 ± 28.50 ^b
	b2 (2%)	240.50 ± 27.50 ^b	242.00 ± 27.00 ^b	244.00 ± 24.00 ^b
Hematocrit (%)	b0 (0%)	31.80 ± 0.10 ^b	31.80 ± 0.00 ^b	31.10 ± 0.50 ^{ab}
	b1 (1%)	31.50 ± 0.40 ^{ab}	30.80 ± 0.20 ^a	30.95 ± 0.95 ^{ab}
	b2 (2%)	31.60 ± 0.40 ^{ab}	31.25 ± 0.65 ^{ab}	31.35 ± 0.45 ^{ab}
Hemoglobin (g/dL)	b0 (0%)	11.65 ± 0.05	11.85 ± 0.15	11.60 ± 1.20
	b1 (1%)	11.35 ± 0.35	11.15 ± 0.05	11.45 ± 1.35
	b2 (2%)	12.35 ± 0.35	12.40 ± 0.30	11.75 ± 0.45

Note: Data are presented as mean values ± SEM. Means with different letters in the same column and row are significantly different ($p < 0.05$) between treatments.

The hemoglobin level result showed no interaction and ($p > 0.05$) in the 5 weeks of the administration period. The result showed no significant difference between the use of *M. oleifera* extract and the probiotic *Lactobacillus* sp. on hemoglobin levels. The feed intake result showed a significant difference ($p < 0.05$) in the 5 weeks of the administration period. The lowest feed intake values were found for treating a0b2, which is consistent with the treatments of a2b2, a2b1, a2b1, a1b2, a1b0, and a0b0. The highest feed intake values were found for treating a0b1, which were not significantly different from the treatments of a2b0 and a1b1.

The result of feed conversion ratio showed that there was an interaction ($p < 0.05$) in the 5 weeks of the administration period. The lowest feed conversion ratio values were found for treating a1b2, which is consistent with the treatments of a2b1, a1b1, a1b0, a2b2, and a0b1. The highest feed conversion ratio values were found for treating a0b0, which were not significantly different from the treatments of a0b2 and a2b0.

The result of daily body weight gain showed an interaction ($p < 0.05$) in the 5 weeks of the administration period. The lowest daily body weight gain values were found for a0b0 treatment, which is consistent with the treatment of a0b2. The highest daily body weight gain values were found for treatments of a0b1 and a1b2, which were not significantly different from the treatments of a1b1, a2b1, a1b0, and a2b0. The average performance parameters (feed intake, feed conversion

ratio, daily body weight gain) for treating *M. oleifera* extract and probiotic *Lactobacillus* sp. in broiler chickens are listed in Table 2.

The results of the use of *M. oleifera* extract and the probiotic showed an interaction ($p < 0.05$) on the final body weight in the 5 weeks of the administration period. The average values of final body weight for treating *M. oleifera* extract and probiotic *Lactobacillus* sp. in broiler chickens are listed in Table 3. The lowest final body weight was found in treatment (a0b0), which did not differ from treatment (a0b2). The highest final body weight was found in the treatment (a1b2).

The results of the use of *M. oleifera* extract and the *Lactobacillus* sp probiotic showed that there was an interaction ($p < 0.05$) on IOFC treated hens for 5 weeks of the administration period. The price of broiler meat was IDR 34,000/kg, and the feed price was IDR 10,000/kg. The average values of IOFC for treating *M. oleifera* extract and the probiotic *Lactobacillus* sp. in broiler chickens are listed in Table 3. The lowest IOFCs were found in treatments a0b0 and a0b2. The highest IOFC was found in treatments a0b1, a1b2, and a1b0, which did not differ from treatments a1b1, a2b0, a2b1, and a2b2.

DISCUSSION

M. oleifera is widely recognized as one of the most beneficial plants for various purposes (Gopalakrishnan

Table 2. Average values of performance parameters for treating *Moringa oleifera* extract and probiotic *Lactobacillus* sp. in broiler chickens

Variables	Probiotic <i>Lactobacillus</i> sp.	<i>Moringa oleifera</i> extract		
		a0 (0%)	a1 (1%)	a2 (2%)
Feed intake (g/chick/day)	b0 (0%)	74.00 ± 4.36 ^{ab}	73.33 ± 1.53 ^{ab}	77.00 ± 1.00 ^{bc}
	b1 (1%)	81.00 ± 4.36 ^c	76.3 ± 4.16 ^{bc}	72.00 ± 2.64 ^{ab}
	b2 (2%)	69.33 ± 2.89 ^a	72.00 ± 1.00 ^{ab}	70.33 ± 1.53 ^a
Feed conversion ratio	b0 (0%)	1.72 ± 0.14 ^c	1.50 ± 0.025 ^{ab}	1.58 ± 0.12 ^{bc}
	b1 (1%)	1.52 ± 0.01 ^{ab}	1.49 ± 0.13 ^{ab}	1.44 ± 0.05 ^{ab}
	b2 (2%)	1.55 ± 0.13 ^{bc}	1.35 ± 0.06 ^a	1.52 ± 0.07 ^{ab}
Daily body weight gain (g/chick/day)	b0 (0%)	43.33 ± 5.69 ^a	49.00 ± 2.00 ^{abcd}	49.00 ± 3.46 ^{abcd}
	b1 (1%)	53.00 ± 2.64 ^d	51.67 ± 1.53 ^{cd}	50.33 ± 3.06 ^{bcd}
	b2 (2%)	44.67 ± 3.79 ^{ab}	53.33 ± 3.21 ^d	46.33 ± 1.53 ^{abc}

Note: Data are presented as mean values ± SEM. Means with different letters in the same column and row are significantly different ($p < 0.05$) between treatments.

Table 3. Average values of final body weight and income over feed cost (IOFC)

Variables	Probiotic <i>Lactobacillus</i> sp.	<i>Moringa oleifera</i> extract		
		a0 (0%)	a1 (1%)	a2 (2%)
Final body weight (g/chick)	b0 (0%)	1516.67 ± 196.11 ^a	1711.67 ± 63.73 ^{abcd}	1717.00 ± 113.53 ^{abcd}
	b1 (1%)	1850.33 ± 88.79 ^d	1799.00 ± 56.71 ^{cd}	1757.67 ± 97.79 ^{bcd}
	b2 (2%)	1569.67 ± 134.29 ^{ab}	1860.00 ± 102.31 ^d	1620.67 ± 56.88 ^{abc}
IOFC (IDR)	b0 (0%)	25630.00 ± 5292.24 ^a	32246.67 ± 1288.05 ^b	32624.67 ± 4070.96 ^b
	b1 (1%)	33571.33 ± 2140.03 ^b	31112.67 ± 2022.94 ^{ab}	30360.67 ± 2647.64 ^{ab}
	b2 (2%)	24855.33 ± 4377.88 ^a	34316.67 ± 3097.17 ^b	29549.33 ± 3241.407 ^{ab}

Note: Data are presented as mean values ± SEM. Means with different letters in the same column and row are significantly different ($p < 0.05$) between treatments.

et al., 2016). Some parts of the *Moringa* tree, such as the leaves, clusters, seeds, blooms, fruits, and roots, are used in cooking, whereas others have medicinal uses (Patil *et al.*, 2022). *Moringa* contains a high concentration of phytochemical substances, which endow the plant with significant medicinal characteristics and make it potentially useful for treating various illnesses (Abd Rani *et al.*, 2018). The leaves and seeds of *M. oleifera* contain antioxidants, protein, iron, calcium, ascorbic acid, and vitamin A (Asghari *et al.*, 2015). Other antioxidant compounds include carotenoids, flavonoid vitamin E, and phenolic compounds (Gopalakrishnan *et al.*, 2016).

Disaccharides and other simple polysaccharides are used to produce most prebiotics (Yoo *et al.*, 2012). For instance, the growth of *Lactobacillus* sp. is facilitated by the polysaccharides in mushrooms (Nowak & Greenfield, 2018). Although prebiotics are made up of indigestible carbohydrate fibers, the recognition of the polyphenolic component as a possible prebiotic has only recently emerged during the past ten years (Chen *et al.*, 2022).

The use of *M. oleifera* extract and the probiotic *Lactobacillus* sp. on leucocyte levels in treated hens for 5 weeks resulted in an interaction ($p < 0.05$), according to the study findings (Table 1). The lowest leucocyte values were found in treatments (a0b2), (a1b2), (a2b0), (a2b1), (a1b1), and (a0b1), which did not differ from treatment (a2b2). High leucocyte values were found in the treatment (a0b0). However, these values are still within the normal range of chicken leucocyte values. Normal

leucocyte values are 20–30 $10^3/\text{mm}^3$. The findings of this study differ from Das' research, which found that the usage of probiotics and prebiotics did not significantly affect total leucocyte count (TLC) (Das *et al.*, 2016).

The current investigation results revealed that the dosage of probiotics and *M. oleifera* extract did not significantly change the erythrocyte count (Table 1). This study demonstrated that there was no interaction between the probiotic *Lactobacillus* sp. and the extract of *M. oleifera* on erythrocyte levels in treated hens for 5 weeks. The results of this study agree with research by Das, who explains that the use of probiotics and prebiotics did not show any significant differences in the total erythrocyte count (TEC) (Das *et al.*, 2016). The provision of *Lactobacillus* sp. and *M. oleifera* extract probiotics in this study showed the same results as Mateova's research (Mateova *et al.*, 2008), that the provision of *Lactobacillus fermentum* probiotics did not show a significant difference to the erythrocyte values in the control and treatment ($2.11\text{--}2.29 \times 10^6/\text{mm}^3$). The normal range of erythrocyte values is $2.50\text{--}3.50 \times 10^6/\text{mm}^3$ (Nabi *et al.*, 2022). The results of variance showed that the treatment had no significant effect ($p > 0.05$) on the number of erythrocytes, but there was a tendency for an increase in the number of erythrocytes as the dose of probiotic *Lactobacillus* sp. increased. The lowest erythrocyte values obtained in this study were found in the control treatment ($2.5 \times 10^6/\text{mm}^3$), and the highest erythrocyte values obtained in this study were found in the treatment with 2% *Lactobacillus* sp. ($2.70 \times 10^6/\text{mm}^3$) and the combination of 2% *Lactobacillus*

sp + 1% *M. oleifera* (2.70×10^6 /mm³). This research also correlates with Hidayat's prior findings that probiotics *Lactobacillus paracasei* could improve the hematological profile of broilers. Therefore, *L. paracasei* indirectly contributes to the absorption of feed substances needed for the formation of erythrocytes (Hidayat *et al.*, 2020).

According to the findings, the amount of *M. oleifera* extract dosage and probiotic dose did not significantly impact the total number of lymphocytes (Table 1). Moreover, the results showed that there was no interaction between the use of *M. oleifera* extract and the probiotic *Lactobacillus sp.* on lymphocyte levels in treated chickens for 5 weeks. Our findings were in contrast to those of Kamruzzaman and Owosibo, who explained that the use of probiotics shows a significant difference in the total lymphocyte count. Giving probiotics could reduce the number of lymphocytes in broiler chickens (Kamruzzaman *et al.*, 2005; Owosibo *et al.*, 2013). The normal range of lymphocyte values is 3%–8% (Zahorec, 2021).

These results showed that the probiotic *Lactobacillus sp.* and the extract of *M. oleifera* significantly affected monocyte levels in chickens treated for 5 weeks ($p < 0.05$) (Table 1). The lowest monocyte value was found in treatment (a1b1), which was consistent with treatments (a0b2), (a1b0), (a1b2), (a2b0), (a2b1), and (a2b2). A high monocyte value is found in treatment (a0b0), which is consistent with treatments (a0b1) and (a0b2). The normal range of monocyte values is 3%–10% (Mangaonkar *et al.*, 2021). The Moringa plant (*M. oleifera* Lam.) is one of the plants with high carbohydrate concentrations. The total carbohydrate concentration in the ethanol extract consists of monosaccharides, disaccharides, and oligosaccharides, i.e., gut microflora nutrients. *M. oleifera* leaf extracts containing oligosaccharides, fructo-oligosaccharides, and malto-oligosaccharides also significantly stimulate the growth of LAB organisms. This study indicated that substances from *M. oleifera* could stimulate the growth of bacteria. *M. oleifera* leaves contain various amino acids and micronutrients, including B vitamins. Most Lactic Acid Bacteria (LAB) species, including *L. fermentum*, require various amino acids and vitamins as nutrients (Arumdani *et al.*, 2023; Olagbemide & Philip, 2014).

The findings demonstrated a significant interaction ($p < 0.05$) between the use of *M. oleifera* extract and the probiotic *Lactobacillus sp.* on the granulocyte levels in the treated chickens for 5 weeks (Table 1). The lowest granulocyte level was found in treatment (a0b0), which was not different from treatment (a0b1). High granulocyte levels were found in treatment (a2b1), which was not different from treatments (a2b0), (a2b0), (a2b2), (a1b0), (a1b1), (a1b2), and (a0b2).

Based on the results of the current study, chickens treated with *M. oleifera* extract and the bacterium *Lactobacillus sp.* for 5 weeks exhibited an interaction ($p < 0.05$) in thrombocyte levels (Table 1). The highest thrombocyte value is present for treating a0b0, which differs from all treatments, whereas a low thrombocyte value is in all treatments except a0b0. The normal range of thrombocyte values is 150–350 10^3 /mm³ (Hermann *et al.*, 2020).

The results of the study showed that the use of *M. oleifera* extract and the bacterium *Lactobacillus sp.* on hematocrit levels in treated chickens for 5 weeks was significantly associated ($p < 0.05$) (Table 1). The lowest hematocrit values were found for treating a1b1, which is consistent with the treatments of a2b1, a2b0, a1b2, a2b2, a0b1, a0b2, and a0b2. The highest hematocrit values were found in the treatments of a1b0 and a0b0, which were not significantly different from the treatments of a2b1, a2b0, a1b2, a2b2, a0b1, a0b2, and a0b2. The percentage of hematocrit values was found to be significantly different between treatments. These values are still within the normal range. Increased hematocrit levels are associated with the increased erythrocyte levels. Hematocrit values were positively correlated with erythrocyte size but negatively correlated with fluid concentration in the chicken body. An increase in hematocrit values has little benefit because the viscosity (thickness) of blood will increase, slowing blood flow in the capillaries and increasing the heart's work. In this study, although there was a decrease in the hematocrit value along with an increase in the dose of *Lactobacillus sp.* However, the increase in the dose of *M. oleifera* extract does not have a negative effect on the physiological conditions of poultry because the value is still within the normal range for chickens (22%–35%) (Hidayat *et al.*, 2020). If the values of erythrocytes, hematocrit, and hemoglobin are normal, it shows that the animals are physiologically healthy.

The use of *M. oleifera* extract and *Lactobacillus sp.* showed no significant difference ($p > 0.05$) between treatments (Table 1). The outcomes demonstrated that the probiotic *Lactobacillus sp.* and the use of *M. oleifera* leaf extract did not interact ($p > 0.05$). The findings of this study support Das's research, which found that hemoglobin (Hb) levels were not significantly affected by the use of probiotics and prebiotics (Das *et al.*, 2016). Normal hemoglobin values are 7–13 g/dL (Lee & Kim, 2016). Hemoglobin in erythrocytes functions to carry oxygen and cause red blood cells to appear. The variance results showed that the treatment had no significant effect ($p > 0.05$) on the hemoglobin level. This study's results agree with Hidayat's research, which showed no difference ($p > 0.05$) between controls and the administration of probiotics to hemoglobin values. The hemoglobin value in this study was higher than that in a study in broilers who were given *L. paracasei* 3 mL/day treatment (8.84 g/dL). Hemoglobin is an oxygen transportation device that is located in erythrocytes; therefore, a decrease in the amount of hemoglobin can occur due to a disturbance of erythrocyte formation (erythropoiesis) (Hidayat *et al.*, 2020). Previously, it was explained that the value of erythrocytes in this study for treating *Lactobacillus sp.* was still within the normal range.

M. oleifera contains bioactive ingredients, including flavonoids, phenolic acids, phytosterols, alkaloids, vitamins, natural sugars, organic acids, and minerals (Saini *et al.*, 2016), rhamnose, glucosides, acetyl glucosides, routine sides, malonyl glucosides, isorhamnetin, quercetin, and fatty acids (palmitic, oleic acid, and linolenic) (Amaglo *et al.*, 2010). The optimal extraction method should be rapid, simple, and

economical. The extraction method with 70% solvent ethanol showed that the extract of *M. oleifera* contained chlorogenic acid and isoquercitrin flavonoid and total phenolics (Caicedo-Lopez *et al.*, 2019; Rodríguez-Pérez *et al.*, 2015). *M. oleifera* leaves contain insoluble dietary fiber (28.91%), which is the largest component of total dietary fiber (87.68%) (Caicedo-Lopez *et al.*, 2019). Because the insoluble dietary fiber component cannot dissolve in water, the rate of passage in the intestinal tract is very slow (Abuajah *et al.*, 2015). This statement relates to the research results on using a combination as a feed additive in broiler chickens, indicating an interaction between the probiotic *Lactobacillus sp.* and *M. oleifera* extract. This indicates that the insoluble dietary fiber component in *M. oleifera* is an efficient source of prebiotics for the probiotic *Lactobacillus sp.*

The characteristics of *L. fermentum* probiotics that survived at pH 3.0 suggest that there may be a tolerance to bile salts and gastric juice in the intestinal epithelium of fowl. *L. fermentum* probiotics have a positive effect against the pathogenic bacteria *E. coli*, *S. aureus*, *S. typhimurium*, and *P. multocida* (Zhang *et al.*, 2022). The addition of oligosaccharides to the diet can improve the immune function and antioxidant capacity and improve the intestinal health of broilers (Chang *et al.*, 2022). The health and output of chickens can be enhanced by supplementation with a blend of probiotics and prebiotics (synbiotics) (Park *et al.*, 2013). *M. oleifera* is one of the potential plants that can be used to enhance the immune system, improve the intestinal health of broilers, and reduce the number of *Escherichia coli* in the intestines (Ullah *et al.*, 2022). The essential elements found in *M. oleifera* include protein, amino acids, crude fiber, extract ether, carbs, energy, minerals, vitamins, and polysaccharides (Teteh *et al.*, 2013).

The results of the study showed that the use of *M. oleifera* extract and the bacterium *Lactobacillus sp.* on feed intake in treated chickens for 5 weeks was significantly associated ($p < 0.05$) (Table 2). The lowest feed intake values were found for treating a0b2, which is consistent with the treatments of a2b2, a2b1, a1b2, a1b0, and a0b0. The highest feed intake values were found for treating a0b1, which were not significantly different from the treatments of a2b0 and a1b1. The addition of 1% probiotic *Lactobacillus sp.* increased feed consumption by 9.46% compared with the control because the nutrient absorption process was more optimal than the control without probiotics. This is related to research that shows that the addition of probiotics increases villus height compared with that without probiotics. Another study showed that supplementation with *L. paracasei* significantly affected intestinal tissue morphometry, and overall, the tissue appeared normal. Duodenum and jejunum morphology showed a more significant effect on *L. paracasei* supplementation than the ileum (Gyawali *et al.*, 2022). An increase in the number of nutrients accessible for absorption could increase feed consumption in a0b1, followed by higher body weight gain compared with controls. This is indicated by a better FCR value than that of the control. The lower the FCR value, the better because it is more efficient in feed use.

The results of the study showed that the use of *M. oleifera* extract and the bacterium *Lactobacillus sp.* on feed conversion ratio in treated chickens for 5 weeks was significantly associated ($p < 0.05$) (Table 2). The lowest feed conversion ratio values were found for treating a1b2, which is consistent with the treatments of a2b1, a1b1, a1b0, a2b2, and a0b1. The highest feed conversion ratio values were found for treating a0b0, which were not significantly different from the treatment of a0b2 and a2b0. The addition of 2% probiotic *Lactobacillus sp.* and 1% *M. oleifera* extract improved the FCR value of 27.41% compared with the control. In this study, the decrease in FCR was due to lower feed intake but higher daily body weight gain compared with controls. The results of this study agree with research showing that dietary supplementation with 10^9 CFU/kg *Lactobacillus salivarius* improved feed conversion ratio and body weight (Chen *et al.*, 2015). Microbes of probiotics can reduce the pH of the intestine, improve digestion, and consequently increase the consumption of nutrients, enhance growth performance by improving digestion of protein, carbohydrates, and lipids, and improve feed conversion ratio (FCR) (Al-Otaibi *et al.*, 2023). The research on supplementation with *L. paracasei* showed a decrease in FCR compared with controls, where the FCR for 1-42 days was 1.49 (Wang *et al.*, 2022). The results showed that the use of *M. oleifera* extract and the probiotic *Lactobacillus sp.* on daily body weight gain in treated chickens for 5 weeks was significantly associated ($p < 0.05$) (Table 2). The lowest daily body weight gain values were found for a0b0 treatment, which is consistent with the treatment of a0b2. The highest daily body weight gain values were found for treating a0b1 and a1b2, which were not significantly different from the treatments of a1b1, a2b1, a1b0, and a2b0. In this study, the addition of a combination of 2% probiotic *Lactobacillus sp.* and 1% *M. oleifera* extract increased the daily body weight gain by 23.08% compared with controls. The same results obtained with adding 1% probiotic *Lactobacillus sp.* can also increase the daily weight gain 22.32% compared with the control. The results of this study agree with the research of Gyawali, who explained that supplementation with *L. paracasei* showed that the daily body weight gain for 1-42 days was 65.45 g/day (Gyawali *et al.*, 2022). Other research has shown that probiotics positively improve growth performance, body weight gain, feed conversion ratio, intestinal microflora, and intestinal health in chickens (Xu *et al.*, 2019; Fesseha *et al.*, 2021). The results of this study agree with Al-Ali *et al.* (2023), who showed that the use of probiotics can increase body weight. Giving broiler chicks with 1% probiotic *Lactobacillus acidophilus*, *L. casei*, and 1% *Bifidobacterium* in water for 42 days enhanced the average daily weight gain of chicks, body weight, and average daily feed intake (Zhang *et al.*, 2021).

The findings of this research also correlate with the prior findings of Fesseha *et al.* (2021), who indicated that feeding probiotics improved broiler growth performance. The chickens supplemented with *Lactobacillus sp.* probiotics showed higher body weight than the control. The highest body weight gain (1556.17 g) was observed

in chickens in the 1g probiotic treatment group (Fesseha *et al.*, 2021). Probiotics help the digestive process by increasing the beneficial microbial population, increasing bacterial enzymatic activity, and improving gut microbial balance, all of which have an impact on feed digestion, absorption, and intake (Cao *et al.*, 2013).

In the provision of probiotics, or *M. oleifera* extract and a combination of probiotics and *M. oleifera* extract showed a higher IOFC than the control (IOFC 25630.00 IDR). The use of probiotics and *M. oleifera* extract can increase feed utilization efficiency and broiler body weight, thereby increasing income over feed cost. The findings of this research also correlate with the prior findings of Afsharmanesh *et al.* (2013), who indicated that the use of probiotics is an effective strategy that has recently been increased due to their beneficial impacts on intestinal microbial balance, as well as health and growth performance of broiler chickens, resulting in safe and economical production.

CONCLUSION

Moringa oleifera extract and probiotic *Lactobacillus sp.* have potential as feed additives. The single-use or combination significantly affects broiler chicks without inducing any side effects on normal physiological blood profile. *M. oleifera* extract and probiotic *Lactobacillus sp.* positively affect the growth performance (improve feed intake, decrease feed conversion ratio, increase daily body weight gain, increase final body weight) of broilers and increase IOFC.

CONFLICT OF INTEREST

The authors confirm that there are no conflicts of interest regarding this manuscript.

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