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The Effect of Probiotics Consortium Isolated from Fermented Fish (Budu) on Broiler Performances and Meat Quality

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ABSTRACT

The aim of this study was to explore the effects of the probiotic consortium (*Lactobacillus parabuchneri*, *Lactobacillus buchneri*, *Lactobacillus harbinensis*, *Schieferilactobacillus harbinensis* and *Lentilactobacillus parabuchner*) isolated from fermented fish (*budu)* in Padang Pariaman Regency on the performance (weight gain, consumption of ration and ration conversion ratio) and carcass quality (carcass presented, crude fat, crude protein, cholesterol and cooking loss in meat) of broilers. Completely Randomized Design with 4 replications and 4 treatments was applied using 80 DOC (day-old-chicks). These chicks were divided into 16 cages, each cage having five chicks. The treatments in this study were: PO: control; P1: 0.5mL/L probiotic consortium in drinking water: P2: 1.0mL/L probiotic consortium in drinking water; P3: 1.5ml/L probiotic consortium in drinking water. The amount of probiotics of the consortium applied was 10^8 CFU/mL. The results revealed that broilers given probiotic consortium in drinking water exhibited significant differences ($P<0.01$) in their production performance and carcass quality compared to control. Based on these experiments, it was found that the use of 1.0ml/L (P2) of probiotic consortium in drinking water gave better results in terms of feed consumption (693.01g/head/week), body weight gain (409.21g/head/week), feed conversion ratio (1.69), carcass percentage (83.52%), meat fat (11.43%), meat proteins (25.69%), meat cholesterol (16.50mg/dL) and meat cooking loss (30.48%) than other treatments. In conclusion, the probiotic consortium (*Lactobacillus parabuchneri*, *Lactobacillus buchneri*, *Lactobacillus harbinensis*, *Schieferilactobacillus harbinensis,* and *Lentilactobacillus parabuchneri*) isolated from fermented fish (Budu), which contained $1x10^8$ CFU/mL bacteria in the supplementation of 1.0mL/L in drinking water can improve the production performance and carcass quality of broiler chickens.

Key words: Broiler, Carcass, Meat Quality, Performance, Probiotic Consortium.

INTRODUCTION

Broiler farming is one of the promising businesses to produce meat. In addition, this business also increases protein consumption by the consumers. Broiler chickens have several advantages; they grow quickly and can be harvested in a short time. Genetic superiority of chicks and good rations can result in optimal production performance. The increase in meat production and carcass quality can be achieved by increasing the quality of ration given to livestock and poultry (Sari et al. 2019). In addition to the factors described above, the cage environment also plays an important role in determining the performance of broilers and the profit obtained by farmers. Broilers production is known to experience rapid developments every year. Broiler harvesting age is getting shorter due to their better genetic potential, especially for growth traits (Apata et al. 2020). In the field of animal husbandry, there are many ways to improve the production performance of chickens. In addition to increased nutritional intake, feed supplements in the form of probiotics and organic acids can increase growth rate and improve livestock health (Sari et al. 2019). One way that is often used is the supplementation of antibiotics to

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livestock rations. Antibiotics are usually given to chickens to promote their growth, improve the health and reduce pathogenic microorganisms in the digestive tract (Roth et al. 2019; Tahir et al. 2023). However, their use has now been banned by the U.S. Food and Drug Administration (FDA) because their use creates residues and results in the development of bacterial resistance against antibiotics (Access Science Editors 2017). So, efforts are needed to replace antibiotics with compounds that can maintain the health and production performance of poultry.

According to Muharlien et al. (2020), in the management of broilers, the production performance parameters that must be considered include ration consumption, weekly ration conversion, live weight and body weight gain. Zampigaa et al. (2021) suggested the production of ration efficiency with good growth and with a relative humidity of 60-70%. The increased productivity of chickens can be achieved through the provision of quality feed. Since the quality feed is relatively more expensive, nutritional manipulation is needed to optimize feed costs by maximizing production. One of the measures to increase and maintain the productivity of chickens is through manipulation of nutrition to maximize the supply of essential nutrients according to their genetics, namely by adding feed additives in the form of probiotic supplements. Probiotics are known to have beneficial effects on livestock production performance through increasing the ability of the intestine to digest rations by improving the balance of microflora. Increasing the efficiency of the digestive tract to absorb nutrients, especially proteins in feed, can improve carcass quality and increase meat production in broilers (Sari et al. 2019; Gul and Alsayeqh 2023; Rashid et al. 2023).

The area of West Sumatera has a large number of fermented food products, one of which is fermented fish called Budu. Coastal communities traditionally produce fermented fish, namely in the Sungai Limau area, Padang Pariaman Regency and Airbangis, Pasaman Regency. These Budu products have a distinctive taste and aroma produced during fermentation. Which microbes play a role in producing these aromas and tastes, and which nutrients are metabolized by these microbes has not yet been reported. Several previous studies have revealed that microorganisms isolated from fermented fish (Budu) have a positive impact on livestock. Maslami et al. (2019) have found that lactic acid bacteria isolated from fermented fish (Budu) can produce glutamic acid, which can improve broiler carcass quality in the form of meat colour and aroma. Anggraini et al. (2019) isolated lactic acid bacteria from Budu; these bacteria can produce gamma amino butyric acid (Gaba), which can reduce the effects of stress on broilers in high-density cages. Recent research by Ardani et al. (2023) explored that lactic acid bacteria and yeast isolated from fermented fish (Budu) can increase the digestibility of animal feed. This suggests that microorganisms isolated from fermented fish products native to West Sumatra called Budu have many uses, especially because these microorganisms have potential as probiotics.

A combination of two or more types of microbes having probiotic properties is also called a probiotic consortium. It is time for scientists in the world to look for beneficial microbes for livestock production, such as microbes that have probiotic characteristics, especially probiotic consortiums. It is generally believed that different probiotics can replace antibiotics, the use of which is currently banned in different countries of the world including Indonesia. Previously, Rodrigues et al. (2020) evaluated the efficacy of five probiotic-based formulations in modulating diversity and relative abundance of microbial community in broilers. that Their results showed that the use of different probiotic mixtures may influence gastrointestinal microbiota composition in broiler chickens. Another role of probiotics is to improve health, increase growth performance and produce high-quality poultry products. Similarly, Zhang and Kim (2014) investigated the use of a probiotic consortium consisting of four microbes and reported encouraging results. Based on the explanation given above, the present study was conducted to find the best probiotic consortium dosage that could improve the production performance and meat quality of broiler chickens.

MATERIALS AND METHODS

This research was supervised and was approved by the Research Ethics Committee of the Faculty of Medicine, Andalas University in Padang, West Sumatra, Indonesia.

Animal and Ration

A total of eighty (80) day-old chicks (DOC) of Lohmann MB-202 type, with an average body weight of 45.61±3.46g, and belong to PT Japfa Comfeed, Indonesia, were used in the present study. The commercial ration made by the PT Charoen Phokpand, Indonesia, specifically for starter period of broilers, containing metabolic energy of 3000kcal/kg and crude protein of 23% was used for feeding these chicks.

Experiment Design and Treatments

Completely Randomized Design (CRD) was used in this study with 4 replications and 4 treatments, using 80 DOC which were divided into 16 cages, with 5 DOC in each cage. The treatments in this study were: PO: control; P1: 0.5mL/L probiotic consortium in drinking water: P2: 1.0mL/L probiotic consortium in drinking water; andP3: 1.5mL/L probiotic consortium in drinking water. Probiotic consortium given to the chickens contained $1x10^8$ CFU/mL bacteria.

Preparation of Probiotics Consortium

The probiotics consortium used in the present study was a mixture of five microbes including *Lactobacillus parabuchneri*, *Lactobacillus buchneri*, *Lactobacillus harbinensis*, *Schieferilactobacillus harbinensis*, and *Lentilactobacillus parabuchner* at the ratio of 1:1:1:2:1, respectively. The required amount of probiotics consortium was mixed with drinking water and given to the respective chicks every day for 35 days. Probiotics were previously isolated from fermented fish (Budu). The five probiotics were a collection from the Feed Industry Technology Laboratory, Faculty of Animal Science, Andalas University, Padang. Each probiotic consortium contained $1x10^8$ CFU/mL bacteria. Production of probiotics consortium was carried out using alternative Halal media, as described by Marlida et al. (2022).

Animal Rearing Management

At arrival, the day-old chickens were first weighed to determine their initial body weight, and subsequent weighing was carried out weekly. Experimental chickens were fed ad libitum and given access to drinking water with or without probiotic consortium for 24 hours. The duration of the experiment was 28 days.

Production Performance

Broiler chickens were monitored to record the performance criteria during maintenance which included feed consumption, body weight gain, and feed conversion ratio. Feed consumption was recorded by calculating the total amount of feed given minus the remaining feed. Feed conversion ratio was obtained by calculating the ratio between feed consumption and body weight gain.

Meat Quality

At the end of the 4th week, when experimental chickens were 28 days of age, three chickens from each replicate were randomly selected to test the quality of carcass meat. Chickens were slaughtered by applying the principles of Halal and animal welfare, then the entrails were removed, and the harvest was ready (Falaki et al. 2011). Carcass weight was obtained by weighing the harvest without internal organs, except for lungs and kidneys, head, legs, neck, feathers and blood. Meat fat was measured, as described earlier (AOAC 2019). Cholesterol analysis of broiler breast meat was based on the Liebermann-Burchard method (Kleiner and Dotti 1962). The crude protein content in meat was determined by using the Kjeldahl method (AOAC 1995).

Meat Cooking Loss

The meat sample from breast was used in the cooking loss test. The meat sample weighed ±20g and was in the form of a block with a cross-section size of approximately 2x3cm with the direction of the muscle fibers parallel to the tip of the sample. The sample was put in a plastic bag, the latter was labelled and tightly closed so that water could not enter the plastic bag. Then the meat samples were cooked in a container filled with water at 80^0C for one hour. After cooking, the bags with meat samples were placed in a glass beaker filled with cold water at 10^0C for 15 minutes for cooling. Then, the samples were removed from the plastic bags, dried with tissue paper and re-weighed on an analytical balance (Soeparno 2009). Cooking losses were calculated by using the following formula:

CL (%) = $\frac{B1-B2}{B1}\ X\ 100$ where

B1 = Initial sample weight

B2 = Sample weight after cooking

CL = Value of Cooking Loss

Statistical Analysis

Results the data of this study were analyzed using a one-way analysis of variance (ANOVA). Treatments that showed significant effects (P<0.01) were then tested using Duncan Multiple Range Test (DMRT) for comparison among means. All statistical analyses were performed by using IBM Statistical Package for the Social Sciences Statistics 26.0 version (IBM Corp., NY., USA).

RESULTS AND DISCUSSION

Production Performances of Broilers

Feed Consumption: These experiments showed that broilers receiving rations containing various levels of probiotics consortium had highly significant difference (P<0.01) in feed consumption (Table 1). The lowest feed consumption was recorded in treatment P2 (1.0mL/L of probiotic consortium in drinking water) compared to other treatments (P0, P1 and P3). Further analysis showed that treatment P0 was non-significantly different from P1 (P>0.05) but was highly significantly different (P<0.01) from treatment P2 and significantly different (P<0.05) from treatment P3. Thus, the lowest feed consumption was recorded in the P2 treatment. This is because of the fact that probiotics can compete with pathogenic microbes, which can improve the balance of intestinal microflora and feed digestibility. Astuti et al. (2015) also reported that administration of mixed probiotics can synergize with microflora and indigenous microorganisms in the intestine, which can have an impact on increasing digestibility and increasing production performance. Use of probiotics can enhance the activity of digestive enzymes, including protease, amylase and lipase, to increase availability of food substances for the production of meat (Sjofjan et al. 2015). According to Rehman et al. (2020), probiotic supplementation decreases gastric emptying time, leading to higher feed intake. With probiotic supplementation, the gastrointestinal microbiota is balanced, which is essential for early gut development, and leads to a higher feed intake in broilers during the starter phase.

Similarly, Depson (2012) has stated that probiotics are used as feed supplements to help the digestive system of poultry, to make it easier to digest and increase the capacity of digestion, so that more feed substances are available for growth and production. According to Mountzouris et al. (2010), probiotics can improve health status by stimulating intestinal function, improving the composition of digestive microflora and increasing the absorption of food in the intestines of poultry. Microbial balance in the digestive tract can occur if the composition of microorganisms consists of as much as 15% pathogenic microbes and 85% beneficial microbes (Sjofjan et al. 2015). Fuller and Perdigon (2000) reported that the mechanism of probiotic interaction in the digestive tract is that it creates a balance of microflora in the digestive tract by inhibiting the proliferation of pathogenic microbes. As a probiotic, lactobacillus is suitable for domestic animals, because it can inhibit the growth of pathogens and promote the growth of nonpathogenic bacteria by producing antimicrobial metabolites, thereby improving intestinal microecological environment (Vieco-Saiz et al. 2019). Pathogenic or zoonotic bacteria commonly found in poultry farms include *E. coli*, *Salmonella enterica*, *Campylobacter jejuni* and *Clostridium perfringens* (Tarabees et al. 2019).

Body Weight Gain: Broilers that received rations containing various levels of probiotics consortium in drinking water showed highly significant difference (P<0.01) in body weight gain compared to control group. Weight gain was in line with ration consumption, as the highest body weight gain was recorded in P2 (1.0mL/L of probiotic consortium in drinking water) treatment (Table 1). The probiotic consortium is known to play an important role in improving the performance of the digestive organs by keeping the intestinal microflora in a stable state, stimulating the liver to secrete bile and pancreas to release pancreatic juice which contains various enzymes such as amylase, lipase and protease to improve the digestion of feedstuffs in the form of carbohydrates, fats and proteins. The results of this study are consistent with the report by Timmerman et al. (2006), who evaluated the effect of supplementation of seven Lactobacillus strains (*L. fermentum W227.5, L. sanfranciscensis W205.6, L. bifermentans W204.5, L. sanfranciscensis W208.6, L. reuteri W227.3, L. reuteri W218. 2* and *L. reuteri W223.5)* as probiotics given to broiler chickens on their growth performance. Several previous studies have shown that the use of Lactobacillus multi-strain as a probiotic in poultry can have an impact on production because it can increase growth (Gheorghe et al. 2018, Reuben et al. 2022). According to Reuben et al. (2022), probiotic supplementation in the form of multi-strains is better because it can improve the growth performance of broilers better than probiotics given in the form of single strains. This is also in line with Timmerman et al. (2004), who stated that multi-strain probiotic supplementation in broilers have a more positive impact on growth performance than a single strain.

Feed Conversion Ratio: Higher efficiency ration was in the form of treatment P2 (1.0mL/L of probiotic consortium in drinking water), where lower feed conversion ratio was seen compared to other treatments (Table 1). Analysis of variance showed that treatments using probiotic consortium in drinking water had a highly significant effect on ration conversion (P<0.01), the effect of treatment P0 was highly significantly different from treatments P1, P2 and P3.

Feed conversion ratio is the ratio between the feed consumed and the body weight gained by an animal or a bird. The feed conversion ratio indicates the efficiency level of ration used. A lower feed conversion ratio shows the higher efficiency value of the ration, and the ration would be more economical than a ration with higher feed conversion ratio. In this study, supplementation of probiotics consortium given in drinking water decreased the feed conversion ratio. This was caused by the improved intestinal histomorphology so that the absorption of nutrients became more abundant. The growth rate and feed efficiency are influenced by the ability of livestock to consume, digest, absorb and metabolize feed nutrients (Sjofjan et al. 2015). Decrease in feed conversion ratio can occur due to an increase in feed energy metabolism, and increased net availability of energy and proteins for deposition in muscles. The positive response to this effect is the improvement in feed efficiency as seen by achieving a low feed conversion ratio value (Pym 2005). Probiotics help in establishing a microbial balance in the digestive tract of the host, which in turn has an impact on the health status of the host animal (Kompiang 2009). In addition, the formation of a balance of microflora in the digestive tract of poultry can optimize the efficiency of feed consumption and feed conversion ratio (Sjofjan et al. 2015).

Meat Quality

***Carcass Percentage*:** We observed that various levels of the probiotic consortium had a highly significant (P<0.01) effect on broiler carcass quality (Table 2). Carcass percentage showed highly significant differences (P<0.01) among treatments, where the best treatment was P2 (1.0mL/L of probiotic consortium in drinking water) with 83.52% carcass which was highly significantly different from other treatments (P<0.01). Based on this research, it appears that supplementation of probiotics consortium in drinking water can increase the percentage of broiler carcasses. This improvement in carcass percentage and live weight of broilers could have been the result of the cumulative effects of probiotic microbial actions such as increasing digestive enzyme activity, maintenance of beneficial microbial populations and neutralizing the effects of feed toxins in the intestinal environment to improve digestion and nutrient utilization (Chen et al. 2013). Several previous studies have also shown that feed supplementation of probiotics can increase the percentage of meat carcasses. Our results are in line with those of the previous studies which showed that probiotic supplementation had a significant effect on live carcass yield, weight gain, immune response, and prominent pieces of meat (Soomro et al. 2019). Swain et al. (2012) reported a carcass yield of 66.97% with probiotic yeast supplementation of 1.0g/kg of feed, while according to Aguihe et al. (2017), probiotic supplementation resulted in a carcass yield of 76.73%.

Similarly, a report by Ghasemi-Sadabadi et al. (2019) also showed a carcass yield of 76.50% in the presence of PrimaLac probiotic supplementation. The addition of probiotics increased carcass yield in broilers as previously reported by Kaushal et al. (2019) and Ghasemi-Sadabadi

Table 1: Feed consumption, body weight gain and feed conversion ratio of broiler rations with different probiotic consortium dosages

Parameters	Treatments			
	P0	P1	P2	P3
Feed consumption (g/head/week)	719.33±7.26^{a}	715.00±13.36^{a}	693.01±9.61^{b}	713.09±6.07^{ab}
Body weight gain (g/head/week)	374.76±0.98^{a}	390.00±4.80^{ab}	409.21±6.73^{c}	406.45±1.98^{abc}
Feed Conversion Ratio	1.92±0.02^{a}	1.83±0.03^{b}	1.69±0.02^{c}	1.75±0.01^{d}

P0: control; P1: 0.5mL/L probiotic consortium in drinking water: P2: 1.0mL/L probiotic consortium in drinking water; P3: 1.5mL/L probiotic consortium in drinking water. Values (mean±SD) with different superscripts in a row differ highly significantly from one another (P<0.01).

Table 2: Broiler carcass characteristics (carcass presentage, meat fat, meat protein, meat cholesterol and cooking loss) with different probiotic consortium dosages

Parameters	Treatments			
	P0	P1	P2	P3
Carcass presentage	73.89±0.64^{a}	74.58±1.27^{b}	83.52±0.96^{b}	76.14±1.22^{b}
Meat fat (%)	2.64±0.44^{a}	1.05±1.29^{b}	1.18±0.96^{b}	1.50±0.92^{b}
Meat proteins (%)	22.72±0.94^{ab}	26.91±0.77^{a}	25.69±0.88^{a}	24.87±1.23^{a}
Meat cholesterol (mg/dL)	66.51±3.41^{a}	41.84±1.08^{b}	16.50±1.04^{c}	16.47±0.94^{c}
Cooking loss (%)	31.94±0.41^{a}	31.87±0.60^{a}	30.48±0.83^{b}	31.01±0.50^{ab}

P0: control; P1: 0.5mL/L probiotic consortium in drinking water: P2: 1.0mL/L probiotic consortium in drinking water; P3: 1.5mL/L probiotic consortium in drinking water. Values (mean±SD) with different superscripts in a row differ highly significantly from one another (P<0.01).

et al. (2019). Ghasemi-Sadabadi et al. (2019) also reported that supplements from probiotics consortium (*L. acidophilus, L. casei and B. thermophilum*) had a significant effect on the yield of carcasses, thighs yield, and abdominal fat in roosters and Ross 308 hens. In contrast, Qorbanpour et al. (2018) reported that multi-strain probiotics (*L. acidophilus, L. casei, E. faecium* and *B. thermophilum*) had non-significant effect on carcass, breast and thigh weights. Our findings revealed that addition of probiotics effectively increased carcass yield and reduced the level of meat fat in broilers. Therefore, it seems that the use of probiotics improves digestion and absorption through increase in gut microbes' population and improved gut health, and the absorption of balanced nutrients increases, which decreases the meat fat.

***Meat Fat, Proteins and Cholesterol Contents*:** The contents of fat, proteins and cholesterol in broiler meat following the addition of probiotics consortium in drinking water are shown in Table 2. The meat fat and meat cholesterol contents of broilers were significantly lower in the P2 and P3 treatments than in other treatments. Moreover, the treatments of probiotics (P1, P2 and P3) increased the protein contents of meat. In this study, an increase in meat protein levels due to the supplementation of probiotics increased the efficiency of feed due to the increase in absorbed nutrients. In addition, probiotics can produce protease enzymes which degrade proteins into amino acids, the latter can be easily absorbed by the body (Sjofjan et al. 2015; Reuben et al. 2022). Broiler chickens always have excessive fat accumulation, especially in the stomach area, which can result in reduced carcass yield. Administration of probiotics has been reported to reduce fat contents and improve broiler carcass properties (Sugiharto et al. 2018). There was a reduction of meat fat and cholesterol levels in broiler due to the probiotics, as the latter can synthesize lipase enzymes. Liong and Shah (2005) have stated that *Bacillus* sp. can synthesize lipase enzymes that convert fat into fatty acids and triglycerides, resulting in lower meat fat and cholesterol levels in the body. According to Kumar et al. (2012), lactic acid forming bacteria are also able to produce bile salt hydrolase (BSH) enzymes in the digestive tract so that these enzymes can reduce cholesterol levels. These enzymes function to break down bile acids into deconjugated bile acids in the form of free cholic acid which is slightly absorbed by the small intestine and excreted through feces, so this can lower cholesterol levels in meat or blood. In addition, reduced levels of free fatty acids in feed can also lead to decreased cholesterol because free fatty acids are converted to acyl CoA and then acetyl Co-A which ultimately becomes the main precursor for cholesterol formation (Lovita 2005). Our findings show that the higher the dose of probiotic consortium, the greater is the reduction in cholesterol, but in this study, there was non-significant difference (P>0.05) between the P2 and P3 treatments. Both treatments showed lower cholesterol levels than the control and P1.

Cooking Loss: The results regarding cooking loss for broiler chicken meat are shown in Table 2. Supplementation of the probiotic consortium had a significant (P<0.01) effect on cooking loss of meat. It decreased significantly in the treatments of various dosages (P1: 31.87%, P2: 30.48%, P3: 31.01%) compared to control (31.94%). Supplementation of various dosages of probiotic consortium caused significantly different reductions in meat cooking loss. These results show that supplementation of probiotics consortium in drinking water can decrease the cooking loss of broiler meat. These results are in line with the report of Mohammed et al. (2021), who reported that the probiotic *Bacillus subtilis PB6* in the diet increased water holding capacity and reduced cooking losses in broiler leg meat; it also increased the moisture retention and tenderness of the meat. Mohammed et al. (2021) also reported the value of meat cooking losses as 15.75% with *Bacillus subtilis PB6* probiotic supplementation. Increased water holding capacity of meat could be another benefit of probiotic supplementation, as the water holding capacity of meat is affected by the amount of muscle proteins (Filho et al. 2017). Changes in fatty acid composition are associated with increased water holding capacity and contribute to meat tenderness (Yang et al. 2010). According to Apata et al. (2020), meat samples from poultry kept on feed supplemented with probio enzymes at the dose of 0.15% had a lower cooking loss of 10.34%, while treatment with antibiotics had a higher meat cooking loss of 26.70%. This indicates that probiotics have the ability to retain more moisture or juices in the meat than antibiotics. Meanwhile, the research findings of Khalil et al. (2021) showed 14.57% cooking losses in the presence of Protexin probiotic supplementation at high livestock densities. Popova (2017) observed that feeding a diet with probiotics showed an increase in the quality of poultry meat due to an increase in gut microbiota and a reduction in intestinal pathogenic bacteria, which in turn improved the health status, production performance and meat quality of poultry.

Conclusion

Based on this research, it can be concluded that the probiotic consortium (*Lactobacillus parabuchneri*, *Lactobacillus buchneri*, *Lactobacillus harbinensis*, *Schieferilactobacillus harbinensis*, and *Lentilactobacillus parabuchneri*) isolated from fermented fish (Budu) given in drinking water can improve the production performance and carcass quality of broiler chickens. The supplementation of 1.0mL/L (P2) of probiotics consortium in drinking water showed better feed consumption (693.01g/head/week), body weight gain (409.21g/head/week), feed conversion ratio (1.69), carcass percentage (83.52), meat fat (1.43%), meat proteins (25.69%), meat cholesterol (16.50mg/dL) and cooking loss (30.48%) compared to other groups.

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Conflicts of Interest

All authors certify that there is no conflict of interest in the work presented in this manuscript.

Authors' Contributions

MKS and YM participated in all stages of the research, namely the research design, the conduct of the experiment, sample analysis, data analysis, writing, and editing of the manuscript. Harnentis participated in conducting the investigation and working in the lab, Jamsari was responsible for data analysis. LRA wrote and finalized the manuscript. All authors reviewed and approved the manuscript.

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