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Research Article

Utilization of Fermented Soybean Milk Waste with *Lactobacillus casei* T22 in Broiler Rations

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Abstract | In Indonesia, the production of soybean milk generates significant amounts of waste, primarily in the form of soybean milk residue, which is often discarded or underutilized despite its potential as a cost-effective feed ingredient for livestock. Therefore, this study aims to determine how broiler performance is affected by the inclusion of fermented soybean milk waste (FSMW) with *Lactobacillus casei* in the rations of broiler chickens. One hundred broilers of the DOC *Lohmann* strain MB-202 Platinum were used in this study. The cages consisted of 25 units of box cages, each measuring 80 x 80 x 60 cm and housing 4 chickens. Each cage was equipped with feeding and drinking areas. The study used a completely randomized design (CRD) experimental method with 5 treatments and 5 replications, consisting of R1 (control), R2 (20% FSMW), R3 (25% FSMW), R4 (30% FSMW), and R5 (35% FSMW). Parameters measured included feed consumption, body weight gain (BWG), feed conversion ratio (FCR), body weight, carcass percentage, and abdominal fat percentage. The study results indicated that the inclusion of FSMW in rations had a highly significant effect ($P < 0.01$) on feed consumption, BWG, and body weight, but no significant effect ($P > 0.05$) on feed conversion, carcass percentage, and abdominal fat percentage. In conclusion, soybean milk waste fermented with *L. casei* can be included up to 30% in broiler rations.

Keywords | Soybean milk waste, *L. casei*, Fermentation, Performance, Broiler

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INTRODUCTION

In 2022, the agricultural area for soybeans reached 12 hectares, and soybean production in West Sumatra increased from 7 tons in 2021 to 19 tons, marking a 12-ton increase within a year (BPS Sumbar, 2022). Currently, the availability of soybean milk waste (SMW) is abundant due to the growing number of home-based soybean milk industries, driven by increased public awareness of healthy living. The production of soybean milk in Padang has risen annually. According to the West Sumatra Industry Office (2013), there were approximately 20 home-based industries in 2013, a number that increased to 34 by 2020. This growth is attributed to the recognition of the health benefits of consuming soybean milk, which is high in protein and contains isoflavones that can reduce blood cholesterol levels (Koswara, 2006). The demand for soybean milk waste has also increased alongside the rising demand for soybean milk. Approximately 25% of soybean milk waste (SMW) is generated during soybean milk production. Therefore, SMW needs to be utilized as a feed ingredient, particularly for poultry.

Soybean milk waste can be included in animal feed rations due to its relatively high nutritional content, including crude protein (24.76%), crude fat (2.86%), crude fiber (18.15%), ash (2.96%), calcium (0.087%), and phosphorus (0.053%) (Ciptaan *et al*., 2021). Despite its high protein content, the utilization of soybean milk waste in broiler feed is limited, with only 6.2% being effectively used (Mirnawati *et al*., 2012). This limitation is due to the presence of phytic acid (2.98%) and cellulose (9.1%) in SMW (Ciptaan *et al*., 2021). Poultry, particularly broilers, lack the enzymes necessary to break down high levels of crude fiber into simpler forms that can be absorbed by the digestive tract (Pasaribu, 2018). Samtiya *et al*. (2020) further explain that the digestibility of nutrients is further inhibited by the absence of phytase enzymes in the poultry digestive system, which are needed to degrade phytic acid in soybean milk waste. As a result, broilers can only absorb a small portion of the essential nutrients, including protein, present in SMW. To maximize the utilization of nutrients in SMW by broilers, it is necessary to enhance its digestibility.

To improve the usability of SMW, fermentation using cellulolytic and phytase-producing microbes is required. Ciptaan *et al*. (2018) conducted a study fermenting soybean milk waste with 10% *Aspergillus ficuum* inoculum, a cellulolytic and phytase-producing microbe, for 9 days. This process resulted in increased nutritional content, including crude protein (34.95%), nitrogen retention (62.99%), protease activity (7.76 U/ml), phytase activity (7.49 U/ml), cellulase activity (48.55 U/ml), crude fiber content (11.01%), crude fiber digestibility (58.92%), and reduced phytic acid content (0.11%). As a result, fermented SMW can now be included in broiler rations at levels of up to 25% (Aini *et al*., 2023), and up to 25% in quail feed (Ciptaan *et al*., 2024).

Srifani (2023) isolated lactic acid bacteria with cellulolytic and phytase-producing properties from decomposed SMW. These bacteria were sequenced and identified using 16S rRNA, revealing the presence of *Lactobacillus casei*. This bacterium exhibited enzyme activities, including cellulase (17.69 U/ml), phytase (22.82 U/ml), and protease (4.09 U/ml). *L. casei* is known for its ability to produce essential enzymes such as phytase, cellulase, and protease. Phytase plays a role in breaking down phytic acid, thereby increasing mineral availability; cellulase aids in degrading crude fiber into simpler sugars; and protease helps break down proteins into more absorbable peptides and amino acids (Wood and Holzapfel, 1995; Gobbetti *et al*., 2019). Mirnawati (2025) conducted an experiment by fermenting soybean milk residue (SMW) combined with *Indigofera* leaf meal (ILF) as a substrate using *L. casei*, a cellulolytic and phytate-degrading bacterium, for four days. The results obtained from the substrate combination of 70% SMW + 30% ILF were as follows: cellulase activity of 17.16 U/mL, phytase activity of 15.13 U/mL, protease activity of 10.72 U/mL, crude protein content of 36.42%, crude fiber content of 11.5%, crude fat content of 2.48%, nitrogen retention of 64.19%, phytic acid content of 5.79%, crude fiber digestibility of 58.02%, and metabolizable energy of 2682 kcal/kg (under publication).

Given the high nutritional content of soybean milk waste (SMW) fermented with *L. casei*, it is essential to conduct experiments to evaluate its potential as an additive in broiler rations. Therefore, the current study was designed to explore the effects of various levels of fermented soybean milk waste (FSMW) on the performance parameters of broilers.

MATERIALS AND METHODS

Study site

This research was conducted from September 2024 to October 2024 in field laboratory at Animal Science Faculty, University of Andalas.

Research materials

This research used 100 DOC broiler *Lohnmann* strain MB-202 Platinum from PT. Japfa Comfeed Indonesia, without separating males and females (unsex). As many as 25 box cage units are used in the experiment. Each unit measures 80 x 80 x 60 cm, houses 4 chickens, was equipped with a feeder, drinker and 60watt incandescent lamp (for heating and lighting). Other equipment used were digital scales, buckets, plastic, brooms, excreta containers and ration waste containers.

Research design

In order to conduct this research, an experimental approach comprising four ration treatments with five repetitions each was used in a completely randomized design (CRD). R1 = 0% FSMW, R2 = 20% FSMW, R3 = 25%, R4= 30%, and R5= 35% FSMW were the treatments that were applied.

The ration used was prepared with a crude protein content of 22% and metabolic energy of 3000Kcal/kg (Soeharsono 1976). Table 1 displays the percentage composition of feed materials in the treatment ration, while the chemical composition and metabolic energy (kcal/kg) level in the ration are shown in Table 2.

Research implementation procedures

Bacterial revitalization

Bacterial revitalization was performed by culturing *L. casei* on MRS Agar media. A total of 62 g of MRS Agar was mixed with 1 liter of distilled water (*Oxoid*) and heated until the solution turned slightly yellowish (clear). The cooked MRS Agar was then poured into test tubes, sealed with cotton and aluminum foil, and autoclaved at 121°C and 1 atm pressure for 15 minutes. The test tubes were tilted and cooled until the media solidified. The solidified media were then inoculated with *L. casei* and incubated at 37°C for 48 hours.

Table 1: Composition of feed ingredients (%) of treatment ration.

Feed ingredients (g/kg)	Treatment rations				
	R1	R2	R3	R4	R5
Yellow corn	52	49	48	50.3	44
Soybean meal	29	12	8	4,5	0.05
Rice brain	7	7	7	3.5	9.45
Fish flour	11	11	11	11	11
FSMW	0	20	25	30	35
Coconut oil	0.5	0.5	0.5	0.2	0
Vitamin-mineral mixture	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Table 2: Chemical composition of treatment rations.

Content					
Crude protein(%)	22.12	22.04	22.10	22.28	22.35
Crude fat (%)	2.85	2.89	2.90	2.54	2.48
Crude fiber (%)	4.52	5.42	5.64	5.51	6.35
Calcium (%)	0.77	0.71	0.69	0.67	0.67
Phosphorus (%)	0.52	0.49	0.49	0.44	0.51
Metabolic energy (kcal/kg)	3010.48	3037.06	3041.82	3058.75	3002.68

Inoculum preparation

The inoculum of *L. casei* was prepared using 100 g of bran as the substrate. Distilled water (70 mL) was added, and the mixture was sterilized in an autoclave at 121°C and 1 atm pressure for 15 minutes. After cooling to room temperature (24°C), the bacteria were diluted in a test tube with 7 mL of Brook solution and inoculated into the bran substrate. The mixture was then incubated for 72 hours. After incubation, the fresh inoculum was ready for use.

Substrate preparation

The substrates used were SMW, bran, and ILP, prepared according to the treatment compositions (A1: 90% SMW + 10% ILP; A2: 80% SMW + 20% ILP; A3: 70% SMW + 30% ILP). Each treatment was weighed (100 g) and placed in 15 x 25 cm polypropylene bags. Distilled water (70 mL) was added, and the substrates were sterilized in an autoclave at 121°C and 1 atm pressure for 15 minutes. After sterilization, the substrates were cooled to room temperature and subjected to fermentation.

Fermentation of SMW

The sterilized substrates were inoculated with 10% *L. casei* inoculum (Mirnawati *et al.*, 2019) and incubated according to the treatment durations (B1: 2 days; B2: 4 days; B3: 6 days). After fermentation, the products were harvested, and enzyme activities were measured in their fresh state. The fermented products were then oven-dried at 50–60°C until a constant weight was achieved, ground, and analyzed for nutrient content.

Parameters measurement

Feed intake (FI), and body weight (BW), was measured weekly to calculate the body weight gain (BWG), and feed conversion rate (FCR). At the 42 days age, broilers were slaughtered and percentage of carcass (PC), nitrogen retention (NR), and abdominal fat percentage (AFP) was calculated. Nitrogen retention was calculated as the ratio of N intake to the amount of N retained in the body during a particular period.

Statistical analysis

All data was analyzed statistically by applying One-Way analysis of variance (ANOVA) according to method of Steel and Torrie (2002). Any difference between treatments was tested by Duncans multiple range test (DMRT) at $P<0.05$.

RESULTS AND DISCUSSION

Impact of FSMW on feed consumption

Based on the results of the variance analysis (Table 3), it was found that the use of FSMW with *L. casei* up to 35% in the diet had a highly significant effect ($P<0.01$) on broiler feed consumption. The results of the DMRT test showed that feed consumption in treatments R1, R2, R3, and R4 did not differ significantly ($P>0.05$), but was significantly higher ($P<0.01$) compared to the feed consumption in treatment R5. From Table 3, It can be observed that the inclusion of SMWF up to 30% in the diet resulted in feed consumption comparable to that of the control diet (R1), whereas treatment R5 exhibited a decrease in feed intake.

The non-significant difference in feed consumption among treatments R1, R2, and R3 is attributed to the use of fermented products (SMWF) up to 30% in the diet, which was still able to comparable the control diet. This is due to the use of fermented products, which are more palatable and easier to digest compared to their raw materials, as fermentation induces physical changes such as taste, texture, and color, leading to higher feed consumption. This is in line with the opinion of Mirnawati *et al.* (2013), who stated that fermented products are not only easier to digest and have better nutritional content but also offer improved flavor, aroma, and texture compared to their raw materials.

The non-significant difference in feed consumption among treatments R1, R2, and R3 is also due to the inclusion of

SMWF fermented directly with *L. casei*, which was isolated from spoiled soybean milk waste. This fermentation process produces simpler and more digestible compounds. This is consistent with the findings of Sari and Purwadaria (2004), who stated that all end products of fermentation contain simpler and more digestible compounds than their raw materials. Therefore, the inclusion of fermented SMWF can improve digestibility in broilers, resulting in similar feed consumption across treatments. Consequently, the amount of nutrients utilized for tissue formation in the chickens is also similar.

The low feed consumption in treatment R5 is attributed to the use of a diet containing 35% SMWF, which resulted in a high crude fiber content of 6.35%. Crude fiber has a bulking effect, causing the chickens to feel full quickly but consuming less feed. This is in line with the opinion of Rizal (2006), who stated that the maximum limit for crude fiber in broiler diets is 6%. This is further supported by Suciani *et al.* (2011), who stated that broilers cannot digest excessively high crude fiber, which leads to a decrease in the efficiency of nutrient utilization. High crude fiber content accelerates the passage rate of digesta, reducing the digestibility of other nutrients, which are then excreted, resulting in decreased feed consumption.

The average feed consumption of broilers in this study was 740.875 g/bird/week. This result is higher than that of a previous study using fermented soybean milk waste with *Neurospora crassa* and *Aspergillus ficuum*, which reported an average feed consumption of 480.97 g/bird/week (Aini *et al.*, 2023).

Impact of FSMW on broiler body weight

Based on the results presented in Table 3, it is clear that the use of fermented soybean milk waste (FSMW) with *L. casei* up to 35% in the diet had a highly significant effect ($P<0.01$) on the body weight gain of broilers. The results of the DMRT test showed that the body weight gain in treatments R1, R2, R3, and R4 did not differ significantly ($P>0.05$), but was significantly higher ($P<0.01$) compared to the body weight gain in treatment R5. From Table 3, it can be observed that the use of FSMW up to 30% can comparable the performance of treatment R1, while treatment R4 showed a decrease in broiler body weight gain.

The non-significant difference in body weight gain among treatments R1, R2, R3, and R4 is attributed to the use of fermented products in these treatments. Fermented materials have improved quality and nutrient content, as well as a complete profile of amino acids, which can enhance body weight gain. This is consistent with the opinion of Mirnawati *et al.* (2019), who stated that during the fermentation process, complex components are broken down into simpler forms, and fermentation can also improve nutrient quality. As a result, the protein content in the substrate is converted into higher-quality amino acids that are more easily digested by livestock. This is further supported by Sari and Purwadaria (2004), who stated that fermented products contain simpler and more digestible compounds, thereby enhancing the nutritional quality of the product.

The non-significant difference in body weight gain among treatments R1, R2, R3, and R4 is also due to the high nitrogen retention observed in these treatments (Table 1). Higher nitrogen retention is associated with greater body weight gain. This aligns with the findings of Corzo et al. (2005), who stated that there is a relationship between the amount of nitrogen retained in the body of livestock and body weight gain, making nitrogen retention a useful indicator for predicting growth.

The low body weight gain in treatment R5 is primarily due to feed consumption, which significantly influences body weight gain. The less feed consumed, the lower the body weight gain. This is consistent with the opinion of Nurdiyanto et al. (2015), who stated that body weight gain is closely related to feed consumption. Higher feed consumption leads to greater body weight gain, while lower feed consumption results in reduced body weight gain.

Table 3: Means of feed intake, body weight gain, feed conversion rate, live weight, carcass percentage, abdominal fat percentage and nitrogen retention percentage of broilers fed various levels of FSMW.

Parameter	R1 (0% FSMW)	R2 (20% FSMW)	R3 (25% FSMW)	R4 (30% FSMW)	R5 (35% FSMW)	S.E
Feed consumption (g/head)	777.85[a]	754.5[a]	748.14[a]	740.88[a]	601.725[b]	20.7
Body weight gain (g/head)	447.64[a]	422.28[a]	443.94[a]	414.94[a]	346.81[b]	12.37
Feed conversion	1.74	1.8	1.68	1.79	1.75	0.07
Body weight (g/head)	1843[a]	1707.2[a]	1810.4[a]	1686.6[a]	1481.8[b]	66.71
Carcass percentage (%)	74.72	73.30	74.27	72.04	71.19	0.89
Abdominal fat percentage (%)	1.02	1.00	0.92	0.98	1.04	0.07
Nitrogen retention (%)	57.95[a]	52.13[b]	55.02[ab]	56.46[a]	41.05[c]	1.46

Note: Different superscripts show highly significant effects ($P<0.01$); SE= Standard Error; FSMW: fermented soybean milk waste.

The average body weight gain of broilers in this study was 414.94 g/bird/week. This result is higher than that of a previous study using fermented soybean milk waste with *Neurospora crassa* and *Aspergillus ficuum*, which reported an average body weight gain of 238.90 g/bird/week (Aini *et al.*, 2023).

Impact of FSMW on feed conversion

As shown in Table 3, the use of FSMW with *L. casei* up to a level of 35% in the diet had no significant effect (P>0.05) on the feed conversion ratio (FCR) of broilers. From Table 3, it can be observed that the use of FSMW up to 35% was able to comparable the performance of the control diet.

The non-significant effect on feed conversion ratio across treatments R1, R2, R3, R4, and R5 is attributed to the balanced relationship between the amount of feed consumed and the body weight gain achieved in each treatment. High feed consumption was accompanied by high body weight gain, while low feed consumption was accompanied by low body weight gain. Since the feed conversion ratio is a comparison between feed consumption and body weight gain, the FCR remained similar across treatments. This is consistent with the opinion of Lacy and Vest (2000), who stated that the feed conversion ratio is the ratio between feed consumption and the body weight gain achieved over a specific period. A higher FCR indicates that more feed is required to increase body weight per unit of weight, while a lower FCR indicates that less feed is needed to achieve the same. A low FCR signifies efficient feed utilization. This is supported by the findings of Allama *et al.* (2012), who stated that a low FCR indicates good feed efficiency, as it reflects the efficiency with which livestock utilize feed for growth.

The statistical analysis showed no significant differences among treatments. However, the variation in FCR across treatments is suspected to be influenced by several factors, including adequate energy content, sufficient nutrient levels in the feed, environmental temperature, and health conditions. This aligns with the statement by Lacy and Vest. (2000) that environmental factors such as uncomfortable temperatures, limited feed and water supply, management practices, feed quality, stocking density, and disease can influence feed conversion efficiency. The average feed conversion ratio of broilers in this study ranged from 1.74 to 1.80. This result is lower than that of a previous study using fermented soybean milk waste with *Neurospora crassa* and *Aspergillus ficuum*, which reported an average FCR of 2.02 (Aini *et al.*, 2023).

Impact of FSMW on body weight

Based on the analysis of variance (Table 3), the use of soybean milk residue fermented (FSMW) with *L. casei* up to 35% in the ration had a highly significant effect (P<0.01) on broiler body weight. The Duncan Multiple Range Test (DMRT) results showed that treatments R1, R2, R3, and R4 were not significantly different (P>0.05) but were significantly higher (P<0.01) than treatment R5. Table 3 shows that using FSMW up to 30% can comparable the broiler body weight in R1 (control ration), whereas the body weight in treatment R5 was significantly lower.

The insignificant difference in body weight among treatments R1, R2, R3, and R4 was due to the high nutritional quality of the rations containing FSMW fermented with *L. casei*. Fermented products enhance digestibility, allowing FSMW inclusion of up to 30% in the ration to maintain a body weight comparable to the control ration. This is consistent with the findings of Mirnawati *et al.* (2022), who stated that fermented products contain simpler compounds that are more easily digested, thereby improving the nutritional quality of feed ingredients. Sukaryana *et al.* (2011) also added that fermented products have good nutritional content and digestibility, making them easily absorbed by poultry.

Furthermore, the insignificant difference in body weight among treatments R1, R2, R3, and R4 was also influenced by feed intake and weight gain (Table 3), which were not significantly different in these treatments. Similar feed intake and weight gain result in relatively similar body weights. This aligns with the statement by Manullang *et al.* (2016) that broiler body weight is influenced by feed intake and weight gain. Murtidjo (2003) also emphasized that body weight is closely related to feed intake, where increased feed intake leads to higher body weight, and conversely, decreased feed intake results in lower body weight.

The reduction in body weight observed in treatment R5 was due to the relatively high crude fiber content in the ration (6.35%). High crude fiber levels in the diet cause poultry to feel full faster and reduce palatability, leading to a decrease in body weight. According to Rizal (2006), the optimal crude fiber content in poultry rations is limited to 3–6% for broilers and 8% for laying hens. Abdelbasset and Djamila (2008) further explained that an increase in crude fiber content in feed reduces carbohydrate digestion, as crude fiber is difficult for poultry to digest. Therefore, the decline in body weight in treatment R5 was also caused by the high crude fiber content in the ration, reaching 6.35%.

The average body weight of broilers obtained at the end of this study was 1686.60 g/bird. This result is higher than in a previous study that used rations containing fermented soybean milk residue with *Neurospora crassa* and *Aspergillus ficuum*, which produced an average body weight of 1344.36 g/bird (Aini *et al.*, 2023).

Impact of FSMW on carcass percentage

Table 3 exhibited that the use of FSMW with *L. casei* at levels up to 35% in the diet had no significant effect (P>0.05) on carcass percentage. As shown in Table 2, the inclusion of FSMW up to 35% resulted in a carcass percentage comparable to the control diet. The non-significant difference in carcass percentage among treatments R1, R2, R3, and R4 is attributed to the improved nutritional content of the diet due to the fermentation of FSMW combined with *L. casei*. Fermentation enhances the quality of feed ingredients by breaking down complex compounds into simpler forms that are more easily digested by livestock. This is consistent with the findings of Sahid *et al.* (2022), who stated that the fermentation process not only decomposes complex components but also synthesizes essential vitamins and growth factors, such as riboflavin, vitamin B12, and provitamin A. The improved quality of FSMW contributes to maintaining a carcass percentage similar to that of the control diet. This is further supported by Ketaren (2008), who reported that the inclusion of fermented products in broiler diets does not significantly alter carcass percentage.

Additionally, the non-significant difference in carcass percentage among treatments R1, R2, R3, and R4 is also influenced by the similarity in live body weight across these treatments. Carcass percentage is directly related to live weight, as indicated by Haroen (2003), who stated that carcass percentage is strongly correlated with body weight. This is further supported by Imamudin *et al.* (2012), who found that higher live weight results in a higher carcass percentage, and vice versa. The average carcass percentage of broilers obtained in this study over a three-week period was 72.04%. This result is higher than a previous study using fermented soybean milk waste with *Neurospora crassa* and *Aspergillus ficuum*, which reported an average carcass percentage of 67.67% (Aini *et al*., 2023).

Impact of FSMW on abdominal fat (%)

Based on the variance analysis results (Table 3), the use of fermented soybean milk waste (FSMW) with *L. casei* at levels up to 35% in the diet had no significant effect (P>0.05) on the abdominal fat percentage of broilers. As shown in Table 3, the inclusion of FSMW up to 35% resulted in a comparable abdominal fat percentage to the control diet. The abdominal fat percentage was calculated by dividing the abdominal fat weight by the live body weight and multiplying by 100%.

The non-significant effect on abdominal fat percentage across treatments is attributed to the relatively similar energy content in all diets, leading to comparable fat deposition. This aligns with the findings of Hidayat (2015), who stated that energy intake directly influences abdominal fat accumulation in broilers. Additionally, age also plays a role in determining the abdominal fat percentage. Broilers aged 5–6 weeks are still in the growth phase, during which absorbed nutrients are primarily utilized for growth, resulting in minimal fat accumulation. This is in line with Pratikno (2011), who reported that fat tissue in chickens begins to develop rapidly at 6–7 weeks of age, with fat accumulation increasing progressively thereafter.

The average abdominal fat percentage obtained in this study ranged from 0.92% to 1.04%, indicating a more favorable outcome. This result is lower than a previous study using fermented soybean milk waste with *Neurospora crassa* and *Aspergillus ficuum*, which reported an average abdominal fat percentage of 1.23% (Aini *et al*., 2023).

CONCLUSION

Finding from current study suggested that fermented soybean milk waste (FSMW) with *Lactobacillus casei* could be used up to 30% in the diet to sustain the performance of broilers. This is evident from the average feed intake of 740.86 g/bird/week, body weight gain of 414.94 g/bird/week, feed conversion ratio of 1.79 g/bird/week, final body weight of 1686.60 g/bird, carcass percentage of 72.04%, and abdominal fat percentage of 0.98%. Further research is suggested to investigate the effect of FSMW on the health, and immunity of broilers as well as in other avian species.

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NOVELTY STATEMENT

This research studied the effect of fermented soybean milk waste (FSMW) with *L. casei* in ration on broiler performance. From this study, it was known that FSMW with *L. casei* is a fesaible alternative feed ingredient and can be used up to 30% in broiler ration.

AUTHOR'S CONTRIBUTION

MHA: Data conceptualization and curation. HA: Data curation, formal analysis and wrote the original manuscript. M and YM: Validation and writing editor.

Conflict of interest

The authors have declared no conflict of interest.

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