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Broiler's Responses to Containing Fermented Soybean Milk Waste with a Combination of *Neurospora crassa* and *Aspergillus ficuum*

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ABSTRACT

Soybean milk waste (SMW) can be used as feedstuff, especially for poultry. The fermentation using *Neurospora crassa* and *Aspergillus ficuum* enhances SMW usefulness. This study aimed to determine the effect of fermented soybean milk waste (FSMW) with *Neurospora crassa* and *Aspergillus ficuum* in broiler feed for its performance. We used 100 2-days-old broilers without separating males and females (unsexing). The treatment ration was prepared with a balance of 22% CP and 3000kcal/kg ME. This study used a completely randomized block design with five treatments and four replications. The treatment rations were 0, 15, 20, 25, and 30% fermented soybean milk waste. The parameters studied were feed consumption, body weight, weight gain, FCR, nitrogen retention, carcass percentage, abdominal fat percentage, and crude fiber digestibility. The results showed that feed consumption, body weight gain, feed conversion rate, body weight, carcass percentage, abdominal fat percentage, nitrogen retention, and digestibility of crude fiber were non-significantly (P>0.05) different from treatment 0, 15, 20 and 25% of FMSW in broiler feed. Treatment with 30% of FMSW tended to decrease in all parameters. In conclusion, fermented SMW with *Neurospora crassa* and *Aspergillus ficuum* in a 2:3 ratio can be used up to 25% in broiler rations.

Key words: Soybean Milk Waste, Fermentation, *Neurospora Crassa*, *Aspergillus Ficuum*, Broilers.

INTRODUCTION

The high feed cost is one of the main problems faced by farmers, which comprises 70-80% of production costs. It creates an imbalance between operating costs and selling prices. Many imported poultry feed ingredients such as corn, soybean, and fish meal also cause this problem. Efforts to reduce production costs can be made by increasing the availability of feed ingredients by discovering alternative materials. Soybean milk waste is an alternative solution that can be used as feed ingredients in broiler ration.

SMW is a by-product of soymilk processing. Soy milk contains 1.5, 1.8, and 3.4% carbohydrates, lipids, and proteins, respectively, along with various micronutrients (Zhu et al. 2020; Kumari et al. 2021). According to Ciptaan et al. (2018), SMW contains high nutrition, such as crude protein (24.76%), crude fiber (18.15%), crude lipid (2.86%), ash (7.49%), calcium (0.087%), and phosphorous (0.053%). The demand for soy milk is increasing along with increasing public health awareness. Soy milk is a low-cost non-dairy alternative with better functional and nutritional properties. Despite having reasonably high nutritional contents, the use of SMW is only 6.2% in broiler rations (Mirnawati 2012). Fermentation can increase the quality and palatability of soymilk waste.

The fermentation process utilizes microbic enzymes to convert complex organic compounds under aerobic and anaerobic conditions into simple ones (Bartkiene et al. 2015; Mirnawati et al. 2022a) such as carbohydrates into glucose, proteins into amino acids, and fats into fatty acids), thus making them easily digestible by livestock/poultry and reduce anti-nutrient substances (Alshelmani et al. 2017; Ciptaan et al. 2022a). Fermentation using microbes has recently been developed as an economical method to increase the nutritional value of broiler feeds (Yasar et al. 2020; Peng et al. 2022). *Neurospora crassa* is a mold that is not just able to produce cellulase enzymes but also produces carotenoids which play a role in cholesterol reduction (Li et al. 2015). A previous in vitro study by Zhou et al. (2019) showed

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that oligosaccharides from fermented SMW with *Neurospora crassa* had potential probiotic properties. Mirnawati (2012) stated that fermented SMW with *Neurospora crassa* with the substrate (70% SMW + 30% Bran) could increase crude proteins from 27.62 to 32.64%, reduce crude fiber from 13.81 to 10.88% and crude fat from 6.95 to 4.29% with the ME content of 2767kcal/kg, but it just can be used 20% in broiler rations. This problem could have been caused by high phytic acid (2.98%), so the utilization of SMW in poultry ration is limited (Ciptaan et al. 2018).

Phytic acid can form complexes with proteins, thus reducing protein's availability. Phytic acid also reduces the activity of enzymes such as pepsin, trypsin, and amylase. Phytase is a robust cationic chelator and hurts the bioavailability of essential micronutrients such as Ca, Mg, Fe, and Zn (Rousseau et al. 2020; Sharma et al. 2021). Reducing phytate consumption is necessary, especially if there is a potential deficiency of minerals in the diet (Belmiro et al. 2020). Poultry lacks endogenous phytase to hydrolyze phytic acid in their digestive tract. For the hydrolyzed phytate in SMW, another fermentation process is added by using a fungus *(Aspergillus ficuum*). *Aspergillus ficuum* produces high phytase enzymes (Cheng et al. 2012; Shahryar et al. 2018; Alshamiri et al. 2021; Jiao et al. 2021; Saeed et al. 2021; Jamil et al. 2022). Ciptaan et al. (2018) stated that substrate fermentation (80% SMW + 20% Bran) with *Aspergillus ficuum* yields 34.95% crude protein, 62.99% nitrogen retention, crude fiber 11.01%, crude fiber digestibility 58.92%, cellulase activity 33.84U/mL, protease activity 11.10%U/mL and phytase activity 6.08U/mL%.

Fermentation of SMW with a combination of *Aspergillus ficuum* and *Neurospora crassa* with a ratio of 3:2 has already been used by Ciptaan et al. (2018). This combination has better results reported in the form of crude protein (28.25%), crude fiber (13.77%), nitrogen retention (61.16%), crude fat (1.15%), phytate (0.11g/100g), and carotenoids (4012mg/100g) of FSMW, but its use in rations is yet limited. Based on the description above, the present was designed to study the effects of fermented SMW with combination of two molds in broiler rations and probe the nutritional contents of the formulated feeds.

MATERIALS AND METHODS

Ethical Approval and Experimental Birds

The Animal Ethics Committee of Andalas University, West Sumatera, Indonesia, approved the study. This study was carried out on 2-day-old unsexed 100 broiler Cobb chicks procured from PT. Charoen Pokphand Indonesia.

Preparation of Fermented Soybean Milk Waste

Soybean milk waste and rice bran are used as substrates at a ratio of 80:20%, then mixed and sterilized in an autoclave (121°C for 15min). This mixture was placed at room temperature and mixed with 10% inoculum (*Neurospora crassa* and *Aspergillus ficuum* in a ratio of 2:3.

Subsequently incubated for ninth days with a thickness of 2cm (Mirnawati 2012). After the ninth day, the fermented SMW was incubated (for how long and what temperature) and placed in an oven at 60°C until dry. After that, fermented SMW was made into powder by grinding.

Experimental Design

The research design was completely randomized, with five treatments and four replications. Treatments were 0, 15, 20, 25, and 30% of fermented SMW in the ration. Each replicate consisted of five broilers. The experiment lasted for five weeks. The treatment ration was formulated with a balanced ration content of 22% crude protein and 3000kcal/kg metabolic energy (NRC 1994) and also recommended by Scott et al. (1982). The composition of feed ingredients, nutritional content (%), and metabolic energy (kcal/kg) of the treatment ration are presented in Tables 1 and 2.

The following data were collected:

Feed consumption (g/head/week) = The feed given - Leftover feed

Body weight gain (g/head/week) = Final weight - Initial weight

Feed conversion (FCR) = Feed consumption /Body weight gain.

Body weight (gram): Broilers were weighed after fasting 12h and then killed humanely.

Percentage of Carcass (%) = Carcass weight/Body weight x 100%

Percentage of abdominal fat (%) = Abdominal fat weight/Body weight x 100%

Nitrogen Retention

Feed nitrogen retention was determined by reducing the amount of N consumed by N contained in feces and endogenous N, then divided by N consumed, calculated by the following formula: Feed nitrogen retention = N consumed - (N feces - N endogenous) / N consumed x 100%.

Table 1: Feed ingredients composition (%) in the treatment

Ingredients of feed	Treatment ration (Levels of soybean milk waste fermented)				
	0%	15%	20%	25%	30%
Commercial CP 511 ration	12	12	12	12	12
Yellow Corn	52.7	45.1	42.4	39.7	37.0
Soybean Meal	20.3	12.4	9.8	7.2	4.7
FSMW	0	15	20	25	30
Fish meal	14	14	14	14	14
Coconut oil	0.5	1	1.3	1.6	1.8
Top Mix	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100

Table 2: Nutritional content (%) and metabolic energy (kcal/kg) of the treatment ration

Food Substances	Treatment ration (Levels of soybean milk waste fermented)				
	0%	15%	20%	25%	30%
Crude Protein	22.02	22.00	22.00	22.00	22.04
Crude Lipid	3.01	3.33	3.56	3.80	3.94
Crude Fiber	3.08	4.69	5.23	5.76	6.30
Calcium	1.17	1.18	1.19	1.19	1.19
Phosphorous	0.63	0.60	0.60	0.59	0.58
EM	3007.6	3001.6	3006.2	3010.8	3009

Table 3: Effect of FSMW on feed consumption, body weight gain, feed conversion rate, body weight, crude protein, abdominal fat percentage, nitrogen retention and digestibility of crude fiber in broiler.

Parameter	Treatment ration (Levels of fermented soybean milk waste)				
	0%	15%	20%	25%	30%
FC (g/head)	488.86 a	486.84a	483.19a	480.97a	471.13b
BWG (g/head)	247.79a	243.04a	240.73a	238.90a	208.11b
FCR	1.99a	2.00a	2.01a	2.02a	2.27b
BW (g/head)	1386.55a	1363.20ab	1352.14bc	1344.36c	1189.8d
CP (%)	67.37ab	68.21a	68.55a	67.67a	65.62b
AFP (%)	1.34	1.24	1.24	1.23	1.16
NR (%)	56.43a	56.07a	55.71a	55.36a	51.76b
PDF (%)	50.67a	49.59a	49.03a	48.95a	43.85b

Values bearing different alphabets in a row differ significantly ($P<0.01$). FC=Feed Consumption, BWG=Body Weight Gain, FCR=Feed Conversion Rate, BW=Body Weight, CP=Crude Protein, AFP=Abdominal Fat Percentage, NR=Nitrogen Retention, and DCF=Digestibility of Crude Fiber.

Digestibility of Crude Fiber Feed

Digestibility of crude fiber was determined by reducing the amount of crude fiber consumed with crude fiber contained in the feces, with the following calculation:

Digestibility of crude fiber feed = Consumption of crude fiber - Crude fiber of feces/consumption of crude fiber x 100%

Statistical Analysis

Data thus collected were analyzed with analysis of Variance (ANOVA) through Statistics Systems Analysis (SAS, 1986). Duncans Multiple Range Test (DMRT) was used to determine treatment differences (Steel and Torrie 1991).

RESULTS AND DISCUSSION

The effect of fermented soybean milk waste (SMW) ration treatment with *Neurospora crassa* and *Aspergillus ficuum* (FSMW) is shown in Table 3. The feed consumption (FC), body weight gain (BWG), feed conversion rate (FCR), body weight (BW), carcass percentage (CP), abdominal fat percentage (AFP), nitrogen retention (NR), and digestibility of crude fiber (DCF) were non-significantly different ($P>0.05$) from treatment 0, 15, 20and 25% of FMSW in broiler ration. At the same time, treatment with 30% of FMSW tended to decrease in all parameters.

Feed Consumption (FC)

There was a non-significant ($P>0.05$) difference of treatment with 0, 15, 20, and 25% FSMW on the consumption of broiler rations because SMW fermented with *Neurospora crassa* and *Aspergillus ficuum* probably increased the digestibility of various nutrients and palatability of feed thus livestock prefer this type of ration. These results were in accordance with what was conveyed by Behera et al. (2016), who stated that fermentation could improve the quality and nutrients, for example, amino acids, nitrogen, and crude fiber, and also increases the palatability of feed (Shahowna et al. 2013; Mirnawati et al. 2019).

The average feed consumption obtained by FSMW treatment up to the 25% level ranged from 471.13-488.86g/head/week. These results were lower than the reported consumption of a ration of fermented palm kernel cake with *Sclerotium rolfsii* up to 25% level and obtained average consumption of 2296.36g/head or 459.3g/head/week (Mirnawati et al. 2018).

A decrease in feed consumption with 30% FSMW could be the color of the treated ration as treatment R5 had the darkest color, so broilers less preferred it compared to other treatments. The fermentation process caused the darker color with blackish brown *Aspergillus ficuum* fungi, which dominates more than the *Neurospora crassa* in orange while making the product. Situmorang et al. (2013) stated that the dark color of the ration causes a decrease in palatability because chickens prefer bright-colored rations. According to Borrelli et al. (2017), a decrease in feed intake can be affected by the dark color and undesirable flavor of feed.

Body Weight Gain (BWG)

Fermented SMW with a level of 25% in the feed showed the same BWG as in the control group. It was also observed that the increase of SMW to 30% rendered decreased body weight; this could be due to the low nitrogen retention resulting in lower body weight gain. These findings were in accordance with the results stated by Kidd et al. (2016); they reported the influence between nitrogen detected in livestock on body weight gain, so the growth can be potentially predicted by calculating nitrogen retention.

Decreased weight gain in broiler chickens at 30% SMW could be due to decreased consumption of feed, thus resulting in lower body weight gain. These results were in accordance with the findings of Niu et al. (2017) and Mirnawati et al. (2020). According to them, ration consumption affects body weight gain, and if feed consumption is disturbed, livestock growth will not be optimal.

The average body weight gain obtained using 25% FSMW ranged from 208.11 to 247.79g/head/week. These results were lower than previously reported by Mirnawati et al. (2018). They used fermented palm kernel cake with S*clerotium rolfsii* at a 25% level, resulting in an average body weight gain of 257.7 to 288.5 g/head/week.

Feed Conversion Rate (FCR)

The feed conversion ratio was not significantly ($P>0.05$) different in 0, 15, 20, and 25% treatments which could have occurred as feed consumption and weight gain did not have a significant difference. This was in accordance with the opinions expressed by Stefanello et al. (2015), who stated that high or low FCR is influenced by feed consumption and weight gain.

FSMW to 30% level in feed showed non-significant (P>0.05) FCR difference among all treatments. The use of 30% FSMW could lower feed consumption and BWG ultimately affecting FCR as explained by Truong et al. (2017).

Body Weight

Using 0, 15, 20, and 25% FSMW in the broiler feed rendered the same body weight. Fermented products have good quality and digestibility (Yeniçeri et al. 2021; Mirnawati et al. 2022; Ciptaan et al. 2022a, 2022b); this could be a possible reason that the body weight matched that of the control group. Low body weight using 30% FSMW could be due to low nitrogen retention. This is supported by the findings of (Gomide et al. 2011) that the amount of nitrogen detected in the body is associated with weight gain. Therefore nitrogen retention can help predict growth.

Carcass Percentage (CP)

In the present study, the low carcass percentage with the treatment of 30% FSMW could be due to the low weight of the carcass. Londok et al. (2017) explained that carcass percentage is strongly related to body weight and carcass weight. The percentage of carcass obtained in the present study was 67%, which was lower than the 76% reported by Mirnawati et al. (2022) using *Phanerochaete chrysosporium* and *Neurospora crassa* in palm oil sludge fermentation. The difference between the two studies could be that Mirnawati et al. (2022) used different combinations of organisms for the fermentation of palm oil sludge.

Abdominal Fat Percentage (AFP)

Using 30%, FSMW did not show any significant (P>0.05) effect on abdominal fat percentage in broilers as compared to control and other treatment groups. The rations in this study were prepared with iso energy; therefore, accumulated energy in the body was the same, and thus, body fat was formed the same. Fat accumulation occurs due to excess energy consumed. Pratiwi et al. (2016) suggested that the feed's level of energy and amino acids caused the different percentages of abdominal fat.

There was no apparent difference in abdominal fat in 0, 15, 20, 25, and 30% of FSMW treatments as the broilers were five weeks old. At the age of 7-8 weeks, fatty tissue is formed, especially abdominal fat. Pahlepi et al. (2015) said that energy consumption is usually stored in the fat; thus, abdominal fat is increased in chicken. Ciptaan et al. (2021), who used fermented soybean milk waste with *Aspergillus ficuum*, got abdomen fat to be 1.73-1.83.

Nitrogen Retention (NR)

FSMW at 0, 15, 20, and 25% in the broiler rations provided the same nitrogen retention as the control group in the present study. This could be due to the low crude fiber content in the feed so that the utilization of protein is more optimal. While the decrease in nitrogen retention could be due to the high crude fiber in the R5 treatment (30% FSMW), so the low utilization of protein resulted in low nitrogen retention. This was in accordance with the findings of Kaczmarek et al. (2014), who narrated that crude fiber in the ration significantly affects the absorption of other nutrients, such as protein, so that it affects nitrogen retention. The nitrogen retention found in this study was lower than the results reported by Ciptaan et al. (2022). They reported 61.16% nitrogen retention using FSMW with *Aspergillus ficuum* and *Neurospora crassa* with a ratio of 3:2.

Digestibility of Crude Fiber (DCF)

The digestibility of crude fiber was not significantly affected between the first four treatments in the present study. These results were in accordance with the findings of González-Alvarado et al. (2007), who reported that broilers need fiber in feed necessary to stimulate the digestive tract and improve digestion or absorption of nutrients. The low DCF in the R5 treatment (30% FSMW) could be due to the high crude fiber contents in the ration, affecting digestibility. González-Alvarado et al. (2008) stated that high crude fiber in poultry rations could reduce digestibility. The digestibility of crude fiber in the present study was lower than the result obtained by Ciptaan et al. (2022a). They used FSMW with the combination of *A. ficuum and N. crassa* in a ratio of 3:2 and got 58.76% DCF.

Conclusion

In this experiment, fermented soybean milk waste utilizing *Aspergillus ficuum* and *Neurospora crassa* were used in various combinations in broiler rations. Based on the results obtained, it was concluded that a 25% level of SMW, when mixed in broiler feed, gave the best results in the form of feed consumption (480.97g/head/week), body weight gain (238.90g/head/head in a week) and FCR (2.02). The body weight (1344.36g/head), carcass percentage (67.67%), abdominal fat (1.23%), nitrogen retention (55.36%), and digestibility of crude fiber (48.95%) were also better as compared to other levels of SMW.

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Author's Contribution

Harnentis was in charge of supervising the experiment and writing the original script. Qurrata Aini, Kadran Fajrona, Gita Ciptaan and Mirnawati experimented and analyzed the data. Anifah Srifani finalize manuscript.

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