Effect of Thermomechanical Fermented Soybean Meal on Javanese Super Chicken's Performance, Protease, and Blood Metabolic

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Abstract. Soybean meal, feedstuff obtained from soy seed oil extraction, is a common feed ingredient for poultry despite containing antinutritional factors (ANFs), which can alter digestive activity and decrease poultry productivity. ANFs are reportedly eliminable by fermentation protocol. This study identified the effect of thermomechanical Fermented Soybean Meal (FSBM) on Javanese Super Chicken's protease activity, blood metabolism, and growth performance. A total of 90-day-old chicks (DOCs) of Indonesian male Javanese Super Chicken were allotted to three treatment groups: CON (non-FSBM); T1 (FSBM with Bacillus subtilis); T2 (FSBM with *Aspergillus niger*). All groups were replicated six times with five birds each. The study was conducted for 76 days, and all birds were healthy and vaccinated with Newcastle Diseases (ND), Lasota, and Gumboro A. The result indicated that thermomechanical FSBM affected significantly (P<0.05) daily feed intake, average daily gain, feed conversion ratio, total protein, total albumin, and globulin. Protease activity was not affected by the treatments.

Keywords: Antinutritional Factors, *Aspergillus niger, Bacillus subtilis*, Growth, Poultry.

Abstrak. Bungkil kedelai sangat umum digunakan sebagai bahan pakan unggas meskipun faktor antinutrisi (ANF) terkandung pada bungkil kedelai. ANF tersebut dapat berpengaruh terhadap aktivitas pencernaan dan menyebabkan penurunan produktivitas unggas. Penelitian sebelumnya, ANF dapat dihilangkan dengan protokol fermentasi. Penelitian ini memiliki tujuan yaitu untuk mengetahui pengaruh *thermomechanical fermented soybean meal* (FSBM) terhadap aktivitas protease, metabolit darah, dan produktivitas ayam Jawa Super. Sebanyak 90 *day old chicks* (DOC) ayam Jawa Super jantan digunakan dalam penelitian ini. Seluruh ayam yang digunakan dalam penelitian ini adalah ayam yang sehat dan telah divaksin Newcastle Diseases (ND); Lasota; dan Gumboro A. Seluruh DOC ini dibagi menjadi tiga kelompok yang masing-masing mendapat perlakuan (CON; T1; T2). Setiap kelompok terdiri dari enam ulangan (masing-masing 5 ekor ayam). Tepung kedelai yang difermentasi menggunakan *Bacillus subtilis* dan *Aspergillus niger* sebagai formula pakan ayam merupakan perlakuan dalam penelitian ini (CON (non-FSBM); T1 (FSBM-BS): FSBM menggunakan Bacillus *subtilis*); T2 (FSBM-ASP): FSBM menggunakan *Aspergillus niger*)). Penelitian ini dilakukan selama 76 hari. Hasil penelitian menunjukkan bahwa FSBM berpengaruh nyata (P<0,05) terhadap *feed intake*, *average daily gain*, *feed conversion ratio*, total protein, total albumin, dan globulin. Aktivitas protease tidak terpengaruh oleh perlakuan.

Kata Kunci: Faktor Antinutrisi, *Aspergillus niger, Bacillus subtilis,* Growth, Poultry.

Introduction

Soybean meal (SBM), a by-product of soy seed oil extraction (Li et al., 2020), is a protein-rich feed ingredient commonly used in poultry feed industries (Moughan et al., 2014). It has been reported that unfermented soybean contains 20% soluble protein and 34.5% crude protein (Mukherjee et al., 2016), while another study reported a slightly higher crude protein, namely 47.5% (Zhang et al., 2020). According to Mukherjee et al. (2016), crude protein in SBM is affected by the amount of hull and processing method. SBM produced using solvent extraction without removing the hulls results in low crude protein content, while soybean dehulled before the extraction produced SBM with a high crude protein. Frias et al. (2008) stated that soybean meal is a good primary protein source for poultry because it contains high level of crude protein and amino acid, and is relatively economical.

SBM also contains anti-nutritional factors (ANFs) such as antigenic protein, trypsin inhibitor (Shi et al., 2017), and phytic acid (Heng et al., 2022). ANFs can alter the digestive activity, and decrease utilization of nutrition and

metabolic activity, resulting in decreased performance and health of animals (Suprayogi et al., 2022). Limited amount of phytase enzyme is present in monogastric, causing poor absorption of nutrients bound with phytic acid (Purnamasari and Miswar, 2018). Trypsin inhibitor, present in various legumes such as soybean, can alter the function and utilization of protein in digestion system by decreasing the activity of proteolytic digestion enzyme, making animal suffer from amino acid deficiency (Vagadia et al., 2017). Antigenic protein such as glycinin and β-conglycinin (Liu et al., 2019) are main cause of intestinal diseases, such as intestinal injury, which leads to inflammation (Jingting et al., 2020), monogastric low performance production and high cost production (Yacout , 2016).

Endogenous proteases are group of enzymes synthesized by animal digestive tract that hydrolyzes bulky feed protein into small peptides and amino acids (Frias et al., 2008). Protein offered in sufficient amount in animals will foster positive impacts on growth and development (Rezende et al., 2017) as reflected from the high level of final body weight, average daily gain, average daily intake, total protein, albumin, and globulin of piglets Zuo et al. (2015). This study was conducted to fill the gap in available references about effect of thermomechanical fermented soybean meal (FSBM) on Javanese Super chicken's protease activity, blood metabolic, and growth performance.

Materials and Methods

Ethical Approval

The Animal Ethics Committee of Universitas Sebelas Maret, Surakarta approved the protocol of this research (approval reference number 172/UN27.14.1/PT/2023).

Material

This study was carried out in the experimental farm of the Faculty of Animal Science in Universitas Sebelas Maret, Surakarta from March 14th to June 02nd 2023. A total of 90-day-old healthy chicks (DOCs) of Indonesian male Javanese Super Chicken were vaccinated with Newcastle Diseases (ND), Lasota, and Gumboro A. All DOCs were allotted to three feed treatments using the single step fermented soybean meal (Suprayogi et al., 2022) fermented with *Bacillus subtilis* (BS) and *Aspergillus niger* (ASP). The treatments were CON (non-FSBM), T1 (FSBM-BS), and T2 (FSBM-ASP). All groups were replicated six times with five birds each. The powdered *Aspergillus niger* and *Bacillus subtilis* were obtained from Inter University Center, Universitas Gadjah Mada. Feeding treatment was conducted for 76 days to analyze the digestive protease activity, blood metabolic, and growth performance of Javanese Super Chicken. The nutrient content of FSBM-BS and FSBM-ASP is presented in Table 1, the feed formulation in Table 2, and nutrient composition in Table 3.

Table 1. Nutrient content of FSBM-BS and FSBM-ASP

Nutrient composition	FSBM-BS	FSBM-ASP
Dry matter (%)	88.66	88.66
Metabolism energy (Kcal/kg)	2216	2216
Crude fiber (%)	2.55	2.55
Crude fat (%)	5.23	5.24
Crude protein (%)	49.58	49.24
Calcium (%)	0.31	0.31
Phosphor (%)	0.62	0.62
Methionine (%)	0.43	0.43
Lysine (%)	3.08	2.76

Note: FSBM-BS: FSBM using *Bacillus subtilis*; FSBM-ASP: FSBM using *Aspergillus niger*.

Table 2. The feed formulation of CON; FSBM-BS; and FSBM-ASP

Chemical Composition	Proportion (%)		
	CON	FSBM-BS	FSBM-ASP
Yellow Corn	47.62	48.84	48.63
Rice bran	20.00	20.00	20.00
Coconut oil	2.64	2.43	2.47
Soybean meal	21.53	0.00	0.00
FSBM-BS	0.00	20.51	0.00
FSBM-ASP	0.00	0.00	20.69
Fish meal	5.00	5.00	5.00
DL Methionine 99% Evon ®	0.15	0.15	0.15
L-Lysin CJ ®	0.30	0.30	0.30
Top-Mix Medion®	0.50	0.50	0.50
Mineral B12 Medion®	2.00	2.00	2.00
Salt	0.25	0.25	0.25

Note: CON: Control group; FSBM-BS: FSBM using *Bacillus subtilis*; FSBM-ASP: FSBM using *Aspergillus niger*.

Table 3. The feed nutrients composition of CON; FSBM-BS; and FSBM-ASP

Chemical composition	Starter	Finisher		
		CON	SBM-BS	SBM-ASP
Metabolism energy (Kcal/kg)	3051	2950	2950	2950
Crude protein (%)	21.21	19.00	19.00	19.00
Crude fiber (%)	3.28	2.18	2.16	2.16
Ether extract (%)	4.07	7.43	7.22	7.26
Calcium (Kcal/kg)	1.09	1.49	1.49	0.492
Available phosphor (%)	0.43	1.121	1.117	1.118
Methionine (%)	0.50	0.496	0.492	1.486
Lysine (%)	1.10	1.233	1.369	1.308

Note: CON: Control group; FSBM-BS: FSBM using *Bacillus subtilis*; FSBM-ASP: FSBM using *Aspergillus niger*.

Growth Performance

Performance parameters measured in this research were daily feed intake, average daily gain, and feed conversion ratio. Daily feed intake was calculated daily, body weight was measured weekly, and the average daily weight gain was measured by dividing the total weight gain by the total observation days. Feed conversion ratio was calculated by dividing daily feed intake by the average daily gain (Raheem et al., 2023), (Hariono et al., 2023).

Protease Activity and Blood Metabolic

Protease activity was measured based Cupp-Enyard and Aldrich (2008) method. Blood metabolic parameters observed in this study were total protein, albumin, and globulin. Exactly 3 ml of blood was drawn using a syringe and needle from the external pectoralis vein. The blood was stored in a non-anticoagulant tube and submitted to the Universitas Gadjah Mada vet hospital for centrifugation at 720x g for 10 min to obtain serum. The total protein and albumin were measured individually using biuret method and bromocresol green method, respectively (Osofsky, 2022). Globulin was measured by calculating the difference between total protein and albumin (Rezende et al., 2017), (Zuo et al., 2015).

Statistical Analysis

This study performed one way-analysis of variance (ANOVA) using the IBM SPSS Version 21. Tukey's analysis determined any significant differences ($P<0.05$) between the treatments.

Results and Discussion

Protease Activity

The effects of thermo-mechanical fermented soybean meal (FSBM) on protease activity of Javanese Super Chicken are presented in Table 4. Table 4 shows that replacing non-FSBM with thermomechanical FSBM using *Bacillus subtilis* and *Aspergillus niger* doesn't increase the protease activity. This finding was similar to Feng et al., (2007) who reported no significant difference in protease activity of chickens fed on 25% or 100% FSBM (Raheem et al., 2023). Interestingly, FSBM reportedly did not affect the protease activity in piglets (Yan et al., 2022). A higher level of protease activity in chickens fed on FSBM, as reported by Soumeh et al., (2019), was due to the effect of the fermentation process itself which could interfere with the activity of ANFs and improve the digestibility. There were many specific ANFs contained in soybean meal that reduce the nutrient digestibility of chicken. This study assumed specific ANFs which may not be altered by the fermentation process from this protocol. This assumption is aligned with Soumeh et al. (2019) that specific ANFs such as antigenic protein, non-starch polysaccharides (NSP), and phytate are categorized as a heat stable ANFs that remain stable in high temperature and difficult to eliminate by thermomechanical process. Shi et al. (2017) reported that after the thermo-mechanical process, there remains 13-20% antigenic protein. The contributing factors to the efficacy of thermomechanical fermentation are microbes as the inoculants and thermo-mechanical fermentation process, either single or two steps (Suprayogi et al., 2022). Two-step fermentation is a favorable method because it eliminates ANFs more significantly than single step fermentation. Further research is required to identify which particular ANFs is not affected by the process of fermentation.

Biochemical Metabolic

Biochemical metabolic parameters determined in this study were total protein, albumin, and globulin (see Table 5). The total protein, albumin, and globulin in this study are lower than those in previous studies (Rezende et al., 2017; Tóthová et al., 2019), reporting that the total protein, albumin, and globulin were 2.4 g/dl – 3.3 g/dl, 9.4 g/dl -13.7 g/dl, and 1.5 g/dl – 14.3 g/dl, respectively. Soybean meal replaced with thermomechanical fermented soybean meal in this study has brought significant effect ($p<0.05$) on total protein, albumin, and globulin of Javanese Super chicken. It has been reported that fermentation process can reduce the ANF of feedstuff (Adriani et al., 2021) and the total protein and albumin on chicken fed with FSBM increased significantly compared to the chicken fed with non FSBM (Sembratowicz et al., 2020). Raw soybean meal contains ANFs such as trypsin inhibitors that can alter digestibility (Kuenz et al., 2022; Mukherjee et al., 2016; Nualkul et al., 2022). Trypsin inhibitor, in and of itself, inhibits the proteolytic activity by playing a role as the competitive substrate and binding with serine protease to create an inactive complex (Lima et al., 2019). This study has demonstrated that the level of total protein, albumin, and globulin of Javanese Super chicken fed on FSBM fermented with both BS dan ASP meal was significantly higher ($p<0.05$) than those fed on non-FSBM. This study assumed that the fermentation could reduce the ANF contained in soybean meal.

Table 4. Protease activity of Javanese Super Chicken

Treatments	Protease activity (U/g)
CON	0,0017 ± 0,0018
FSBM BS	0,0012 ± 0,00058
FSBM ASP	0,0010 ± 0,00025
S.E.M	0,00024
p-value	P>0,05

Note: CON= control; FSBM BS= FSBM with *Bacillus subtilis*; FSBM ASP= FSBM by *Aspergillus niger*; S.E.M= standard error of the means

Table 5. Biochemical metabolic of Javanese Super Chicken

Variables	Treatments			S.E.M	p-value
	CON	FSBM BS	FSBM ASP		
Total Protein (g/dl)	2.88[a]	3.95[b]	4.17[b]	0.130	P<0.05
Albumin (g/dl)	1.84[a]	2.20[b]	2.11[ab]	0.055	P<0.05
Globulin (g/dl)	1.04[a]	1.75[b]	2.06[b]	0.101	P<0.05

Note: CON= control; FSBM BS= FSBM by *Bacillus subtilis*; FSBM ASP= FSBM by *Aspergillus niger*; S.E.M= standard error of the means; [a, b]Different superscripts in rows indicated statistically significant difference between treatment (P<0.05)

Growth Performance

Growth performance on Javanese Super Chicken in this study was analyzed based on feed intake, average daily gain (ADG), and feed conversion ratio (FCR). The results are presented in Table 6.

From this study, the thermomechanical FSBM with both *Bacillus subtilis* and *Aspergillus niger* significantly (P<0.05) affected growth performance. The highest feed intake was observed in the group of Javanese Super Chicken fed on FSBM-ASP, followed by FSBM-BS group, and control group. A more significant and higher effect (p<0.05) on average daily gain was identified on both FSBM-BS and FSBM-ASP groups than the control group. In terms of feed conversion ratio, no significant differences were observed between the control group and FSBM-BS, but a significant difference (P<0.05). In general, this present showed that thermomechanical FSBM using both *Bacillus subtilis* and *Aspergillus niger* has significantly affected growth performance by increasing feed intake and average daily gain while decreasing feed conversion ratio. This result was similar to that by Feng et al. (2007) that chicken fed on FSBM had higher levels of feed intake and average daily gain, but significantly lower feed conversion ratio. Previous studies have demonstrated that FSBM could increase feed intake of 40 day-old broiler (Raheem et al. 2023; Sembratowicz et al. 2020) and 42 day old broiler (Feng et al. 2007; Soumeh et al. 2019), increase average daily gain of 42 day old broiler (Feng et al., 2007) and decreased feed conversion ratio of 40 and 42 day old broiler (Raheem et al. 2023; Sembratowicz et al. 2020; Soumeh et al. 2019). These results align with previous studies that FSBM fed to 50 day-old piglet (Xie et al., 2022) and 65 day-old piglets (Yan et al., 2022) could significantly increase (p<0.05) the average daily gain and daily feed intake, while decreasing feed conversion ratio.

This study has demonstrated that feeding FSBM to Javanese Super Chickens increased the total blood protein, albumin, and globulin. Also, these chickens showed a higher feed intake and average daily gain and lower feed conversion ratio compared to chicken fed with non-FSBM. It may be due to the activity of *Bacillus subtilis* and *Aspergillus niger* in fermenting the soybean meal. In Indonesia, SBM is a common feedstuff for chicken (Widiyawati et al., 2020) as it contains high protein and amino acids (Saroh et al., 2019).

Table 6. Growth Performance of Javanese Super Chicken

Variables	Treatments			S.E.M	p-value
	CON	FSBM BS	FSBM ASP		
Feed Intake (g/day)	48.65[a]	49.33[a]	50.52[b]	0.190	P<0.05
Average daily gain (g/day)	11.66[a]	14.59[b]	14.52[b]	0.266	P<0.05
Feed conversion ratio	4.28[a]	3.42[b]	3.53[b]	0.090	P<0.05

Note: CON= control; FSBM BS= FSBM by *Bacillus subtilis*; FSBM ASP= FSBM by *Aspergillus niger*; S.E.M= standard error of the means; [a, b]Different superscripts within rows indicated statistically significant difference between treatment (P<0.05)

Ravindran et al. (2014) found that SBM contained 43–48% protein and abundant amino acids that positively affect growth performance. Unfortunately, SBM also contains antinutritional factors (ANFs) which can alter the protein digestibility process in the digestive tract. Fermentation is a frequently used method to eliminate ANFs, improve nutrient availability, decrease fiber, and by extension, increase protein level of SBM. A previous study reported that SBM fermentation was carried out using *Aspergillus niger* (Jannathulla et al., 2018) and *Bacillus subtilis* (Suprayogi et al., 2022). *Aspergillus niger* can significantly ($P<0.05$) increase crude protein and significantly ($P<0.05$) decrease the trypsin inhibitor, phytic acid, tannin, saponin, and crude fiber. Essential amino acids on the FSBM group are higher than that in non-treated soybean meal (Jannathulla et al., 2018). Yao et al. (2021) used *Bacillus subtilis* for fermenting the soybean meal, and reported that it could significantly ($P<0.05$) decrease ANFs, such as glycinin, β-conglycinin, urease, and phytic acid. Similarly, *Bacillus subtilis* for fermenting the soybean meal significantly increased ($P<0.05$) soybean meal crude content while significantly decreasing ($P<0.05$) neutral detergent fiber (Suprayogi *et al*., 2021). The other benefits of using FSBM is the production of organic acid, which plays important roles in improving gut function. By extension, it positively affects the growth performance (Shi et al., 2017).

Conclusions

Soybean meal fermented with *Bacillus subtilis* and *Aspergillus niger* for Javanese Super Chickens diet could increase the level of blood biochemicals, such as total protein, albumin, and globulin of the chickens. The fermented soybean meal increased the growth performance, such as daily feed intake, average daily gain, and decreased feed conversion ratio.

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