Influence of The Dietary Thermo-Mechanically Fermented Soybean Meal on Chicken Meat Quality of Jawa Super Male Chicken

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Abstract. The meat quality of Jawa Super Male Chicken is attributed to the diet treatment offered since the rearing period. This study aimed to evaluate the meat quality of Jawa Super Male Chickens fed on diets made of soybean meal (SBM) fermented with *Bacillus subtilis* or *Aspergillus niger.* The experimental groups were control group without steam conditioning or fermentation (P0), SBM fermented with Bacillus subtilis or SBM BS (P1), and SBM fermented with Aspergillus niger or SBM ASP (P2). Ninety chickens aged 21 days (210 ± 3.69 grams) were assigned to three groups, and replicated six times with five birds. Basal diets were made of corn-soybean with 16% crude protein (CP) and 2,950 Kcal metabolic energy (ME). The results showed significant differences (P<0.05) in the physical attributes (cooking loss, water holding capacity) and chemical properties (fat content) but the collagen and protein content did not differ significantly (P>0.05). The meat pH was within the normal range of 5.90 – 5.93. Conclusively, SBM fermented with *Bacillus subtilis* or *Aspergillus niger* increased meat quality.

Keywords: Meat quality, Soybean meal, Fermentation, Jawa Super Male Chicken, Thermo-Mechanically.

Abstrak. Kualitas daging Ayam Jawa Super Jantan dipengaruhi oleh pakan yang mereka konsumsi selama masa pemeliharaan. Penelitian ini bertujuan untuk mengamati kualitas daging Ayam Jawa Super Jantan yang diberi pakan perlakuan berupa bungkil kedelai (SBM) yang difermentasi dengan *Bacillus subtilis* dan *Aspergillus niger* dan perlakuan kontrol tanpa perlakuan fermentasi bungkil kedelai. Sembilan puluh ekor ayam pada usia 21 hari (210 ± 3.69 gram) dialokasikan ke dalam tiga kelompok yang terdiri dari enam ulangan dengan lima ekor ayam per ulangannya. Perlakukan yang dilakukan meliputi SBM tanpa fermentasi (P0), dua kelompok lainnya difermentasi dengan *Bacillus subtilis* (P1) dan *Aspergillus niger* (P2). Pakan basal menggunakan *corn-soybean* dengan protein kasar (PK) sebesar 16 % dan energi metabolisme (ME) sebesar 2950 Kkal. Hasil penelitian ini menunjukkan perbedaan yang signifikan (P<0.05) pada kualitas fisik (susut memasak, daya ikat air) dan kualitas kimia (kandungan lemak). Sedangkan kandungan kolagen dan protein tidak berbeda nyata (P>0.05). pH daging berada pada kisaran 5.90 – 5.93, yang menandakan bahwa daging masih dalam kondisi normal. Kesimpulan dari penelitian ini adalah pemberian pakan yang difermentasi menggunakan *Bacillus subtilis* atau *Aspergillus niger* dapat meningkatkan kualitas daging.

Kata kunci: Kualitas daging, Bungkil Kedelai, Fermentasi, Ayam Jawa Super Jantan, Thermo-Mechanically.

Introduction

The soybean meal (SBM) is the most popular protein source for the poultry industry due to its suitable amino acid profile for poultry feed (Erdaw et al., 2016). However, like most popular protein source, SBM has antinutritive factors, such as trypsin inhibitor (Erdaw et al., 2016), phytate, non-starch polysaccharides (NSP), and oligosaccharides (Jezierny et al., 2010), which decrease nutrient utilization (Erdaw et al., 2016). This can be addressed through bacterial or fungal fermentation of SBM to enhance nutrient utilization (Mukherjee et al., 2016). Fermentation of SBM with *Aspergillus orizae* for 2 days can improve the crude protein content in SBM and eliminate trypsin inhibitors (Hong et al., 2004).

Chicken meat quality is associated with the food that they consume, and is measured from its physical attributes (pH, tenderness, meat color, cooking loss, and water-holding capacity (WHC)) and chemical attributes (water content, protein, fat, and minerals content) (Gultom et al., 2023). Chicken meat has a good nutrition composition of 74.86 % water, 23.20 % protein, 1,65 % fat, 0.98 % minerals, and calories kcal 114 (Rosyidi et al., 2009). Park et al. (2021) reported that chicken slaughtered at 28 and 34 days contained 75.29 % vs 76.93 % moisture, 23.02 % vs 23.83 % protein, 0.13 % vs 0.16 % fat, and 0.99 % vs 1.17 % ash.

Meat quality could be observed through chemically and/or physically. Chemically, meat quality is determined by the chemical composition of meat (i.e., water content, protein, fat, and collagen). The physical quality is determined through pH, cooking loss (CL), and water-holding capacity (WHC) (Rasyad et al., 2012). Decreasing meat quality could be detected through the physical quality of meat (Kuntoro et al., 2013).

This study aims to observe the meat quality of Jawa Super Male Chicken fed on SBM fermented with bacteria or fungi. The significance of this study is the substitution of SBM with fermented SBM to improve crude protein and meat quality, and decrease trypsin inhibitor.

Materials and Methods

Animals, experimental design, and diet formulation

This research was conducted in a Completely Randomized Design (CRD) in Jatikuwung Experimental Farm, Faculty of Animal Science, Universitas Sebelas Maret, Surakarta Indonesia. Day-old chick (DOC) was obtained from farmers in Surakarta, then sexed and selected upon arrival at the experimental farm.

The research was conducted in a with three treatment and six replications, each with five chickens. The feed treatments were basal feed of soybean meal (SBM) without steam conditioning and fermentation or SBM SS (P0), SBM fermented with *Bacillus subtilis* or SBM BS (P1), and SBM fermented with *Aspergillus niger* (SBM ASP). Chickens were randomly allotted to cages for the treatments. The *Bacillus subtilis* and *Aspergillus niger* were obtained from Inter-University Center Gadjah Mada University, Yogyakarta, Indonesia. The bacteria and yeast are prepared in powder. The chemical composition of the treated feed is presented in Table 1.

All DOCs were reared for 20 days and fed with basal feed, then fed on diet treatments on the 21st day. Feed was provided twice a day at 07.00 a.m. and 4.00 p.m., and drinking water was provided ad libithum. Basal diets were based on corn-soybean with crude protein (CP) of 16% and metabolic energy (ME) of 2,950 Kcal. Chickens are given vitamins (Vita Chicks®) through drinking water during the rearing period. ND Lasota and Gumboro A vaccination was carried out twice according to the vaccination schedule. The chemical composition of feed from the starter to the finisher period is presented in Table 2.

Table 1. Chemical composition of fermented soybean meal

Chemical composition	SBM BS [1]	SBM ASP [2]
Metabolism energy (Kcal/kg)	2216	2216
Crude protein (%)	49.58	49.24
Dry matter (%)	88.66	88.66
Crude fat (%)	5.23	5.24
Crude fiber (%)	2.55	2.55
Lysine (%)	3.08	2.76
Methionine (%)	0.43	0.43
Calcium (%)	0.31	0.31
Phosphor (%)	0.62	0.62

[1−2] SBM BS, Soybean Meal fermented with *Bacillus subtilis* and SBM ASP, Soybean Meal fermented with *Aspergillus niger*.

Table 2. Diet composition of the treatment groups in starter and finisher periods

Chemical composition	Starter	Finisher		
		Control	SBM BS [1]	SBM ASP [2]
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

[1 – 2] SBM BS, Soybean Meal fermented with *Bacillus subtilis* and SBM ASP, Soybean Meal fermented with *Aspergillus niger*.

Fermentation of SBM

The fermentation of SBM was conducted based Suprayogi et al. (2023). Exactly 1 kg SBM to sterilize for 30 minutes. Then, after the SBM temperature reached 30 – 40 °C, 10 g of *Bacillus subtilis* powder (1 x 108 cfu/g) was inoculated to the SBM for 24h at 30 °C. The same process of preparing SBM was conducted for *Aspergillus niger* powder (1 x 10^6 cfu/g). Then, they were inoculated for 48h at 30 °C. The SBM fermentation was harvested and oven dried at 60 °C.

Sample collection

Sample collection for meat quality was obtained from chickens at 60 days of rearing. The chickens were sacrificed according to halal method as prescribed by the fatwa issued by the Indonesian Council of Ulama (MUI, 2009) and cut to a commercial cuts. The meat sample for quality analysis was taken from the breast.

Sample Analysis

Protein, fat, moisture, and collagen content were analyzed using a FoodScan™ Type 7881, Foss Electric A/S Denmark. The preparation and analysis were conducted based on Anderson (2007). The physical assessment analyzed parameters such as pH, Water Holding Capacity (WHC), and cooking loss.

pH Value

The pH value was measured using a pH meter that had been calibrated with a buffer solution at pH 4. Exactly 7.5 g of the ground sample was added with 50 ml of distilled water mixed until homogeneous, then transferred into a measuring cup. The mixture was then measured with a pH meter to read the meat pH (AOAC, 2005). pH testing was carried out using the triplicate method for each sample.

Water Content

A total of 5 g of sample was put into a cup of known weight, then oven dried at 80 °C until the weight was constant. Then, the sample was cooled in a desiccator and weighed.

$$\text{Water content (\%)} = \frac{\text{Initial weight (g) - Final weight (g)}}{\text{Initial weight (g)}} \times 100\ \%$$

Water Holding Capacity

The Water Holding Capacity (WHC) was determined with a by Park et al. (2021) with a slight modification. Exactly 0.3 g sample was measured then pressed with a 35 kg weight. After five-minute interval, the surface area occupied by the meat sample and the area of the surrounding wet region were delineated and quantified. The WHC was calculated using the following formula:

$$\text{WHC (\%)} = \frac{\text{Meat area (mm}^2\text{)}}{\text{Exudation area (mm}^2\text{)}} \times 100$$

Cooking Loss

Cooking loss was measured with a modified method by Soeparno (2009) in Negari et al. (2015). The sample was weighed 50 g, then put into a polyethylene plastic bag and closed tightly. The samples were boiled in a water bath at 80^o C for 30 minutes. Then, the samples were thawed with running water for 10 minutes. The sample was removed from the plastic, dried with tissue paper, and then weighed again. Cooking loss is calculated using the following formula (Soeparno, 2009) :

$$\text{Cooking loss} = \frac{\text{Weight Before Cooking (g)} - \text{Weight After Cooking (g)}}{\text{Weight Before Cooking (g)}} \times 100\ \%$$

Statistical Analysis

The obtained data were subjected to one-way ANOVA using IBM SPSS Statistics 21 software. Any significant difference observed in the parameters was further analyzed using the Duncan Multiple Range Test (DMRT).

Results and Discussion

Physical Quality of Meat

The result in Table 3 showed a significant difference (P < 0.05) between the WHC and the cooking loss. However, the meat pH did not show a significant difference (P > 0.05) across all treatments.

The physical quality of Jawa Super chickens treated with SBM BS or SBM ASP showed a significant difference (P < 0.05) water holding capacity (WHC) and the cooking loss. Meanwhile, the pH values of all treatments showed no difference (P > 0.05).

An increase in the WHC value in treated meat indicates that SBM fermentation using *Bacillus subtilis* (SBM BS) or *Aspergillus niger* (SBM ASP) can bind water in the meat. This is because the treatment reduces the levels of feed anti-nutrients in the form of trypsin inhibitors and phytate so that the protein absorption process becomes more optimal. This causes the body water contained in meat to be bound by protein so that the WHC ability becomes better. Proteins significantly contribute to the preservation of Water Holding Capacity (WHC) by effectively binding water molecules within muscle fibers (Wang et al., 2022).

Different WHC was caused by increased proteolytic enzyme activity due to decreased trypsin inhibitors in the SBM BS and SBM ASP treatments. Proteolytic enzymes cause damage to muscle membranes, prompting ion diffusion into the meat protein. This process causes the replacement of divalent ions (i.e., Mg^{2+} and Ca^{2+}) with monovalent ions in the protein chain (Afrila and Santoso, 2011), enabling the monovalent protein ions to bind water and maintain WHC.

The decrease in the cooking loss value in treatments using SBM BS and SBM ASP was caused by increased WHC capacity, so the amount of water contained in the meat was bound by protein. This causes the amount of water released during cooking to be lower. Meat exhibiting elevated Water Holding Capacity (WHC) retains greater moisture during the cooking process, leading to reduced cooking losses (Bhawana et al., 2023).

Table 3. pH, water holding capacity, cooking loss, and temperature of meat

Variables	Treatments			S.E.M	p-value
	Control	SBM BS	SBM ASP		
pH	5.904	5.901	5.937	0.020	> 0.05
Water Holding Capacity (%)	25.201 ^{a}	30.882 ^{b}	29.430 ^{b}	0.615	< 0.05
Cooking Loss (%)	24.000 ^{b}	21.100 ^{a}	20.600 ^{a}	0.405	< 0.05
Temperature (°C)	28.490	28.410	28.280	0.063	> 0.05

$^{a-b}$ Means within the same column with different superscripts differ (p < 0.05).

The meat pH across treatments was not significantly different (P > 0.05), indicating that the treatment did not have bad impacts on meat quality. The pH value of Jawa Super Male Chicken meat in this study was higher than that of other superior chicken meat, namely 5.4 – 5.8 (Soeparno, 2015) but similar to meat pH of Joper chickens given additional papaya leaf extract *(Carica papaya L.)*, namely around 5.90 – 6.16 (Girsang et al., 2022).

Chemical Composition of Meat

In Table 4, fat content of Jawa Super Male Chicken decreased by substituting unfermented SBM with SBM ASP (P < 0.05). However, treatment with SBM BS did not show a significant difference (P > 0.05) in fat levels. Meanwhile, protein, collagen, and fat did not show significant differences across treatments (P > 0.05).

The chemical properties of chicken meat that were significantly different was fat. In Table 4, fat level decreased in SBM ASP treatment which could be because Aspergillus contained the compound *3-hydroxy-3-methylglutaryl coenzyme A* (HMG-CoA) reductase inhibitors, called Statins, which inhibit the rate-limiting step in cholesterol synthesis (Saleh et al., 2011). It led to a decrease in meat fat. Furthermore, Aspergillus is perceived to have influenced fat deposition in Java Male Chicken by influencing the activities of hormone-sensitive lipase and malate dehydrogenase enzymes in adipose tissues (Mersmann, 1998). The fat content in this study still showed normal levels, namely 1.2 – 12 % (Gultom et al., 2023).

Protein, collagen, and moisture levels were not different across treatments, indicating that the treatments did not affect the overall chemical composition of the meat. The protein levels of 16 – 22% was within the normal range (Gultom et al., 2023) due to the stability formed in the presence of nitrogen pools in cells, which maintain a stable amount of protein and amino acid in the body. This is because protein and amino acids are not stored in the body, so there is a constant protein turnover mechanism. Proteins and amino acids were subject to continuous turnover within the body as they are not stored for extended periods (Swick, 1982). Some proteins are continuously synthesized while other proteins are degraded. Therefore, this mechanism may have caused protein levels in meat to not differ significantly across treatments.

Collagen is the connective tissue associated with meat tenderness (Intarapichet et al., 2008). High collagen in meat makes meat less tender, and a higher collagen content is found in older animals (Purslow, 2005). The characteristics and proportion of cross-links in collagen are the main factors influencing the tenderness and texture of meat (Weston et al., 2002).

Moisture is also an important parameter associated with meat quality. Moisture in meat varies, depending on the type of meat (Kodra et al., 2019). The water content of meat in this study was in the normal range of 65 – 80% (Fathurrohman et al., 2022) but not significantly different. The moisture percentage in meat depends on the type of muscle, type of animal, and pH value. However, in general, lean meat contains more air.

Table 4. Chemical Composition of Meat

Variables	Treatments			S.E.M	p-value
	Control	SBM BS	SBM ASP		
Protein (%)	22.416	22.619	22.636	0.088	> 0.05
Fat (%)	4.084 ^{a}	3.640 ^{a}	2.828 ^{b}	0.171	< 0.05
Collagen (%)	1.250	1.395	1.331	0.072	> 0.05
Moisture (%)	75.173	74.525	75.374	0.212	> 0.05

$^{a-b}$ Values bearing different superscripts within column indicate differences (p<0.05).

Conclusions

This study concludes that replacing unfermented soybean meal with soybean meal fermented with *Bacillus subtilis* or *Aspergillus niger* leads to improved water-holding capacity, reduced cooking loss, and decreased meat fat content. However, the treatments did not change the water content, collagen, protein, and pH level of the meat.

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