Improvement of broiler meat quality due to dietary inclusion of soybean oligosaccharide derived from soybean meal extract

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Abstract. Dietary inclusion of antibiotics as growth promoters (AGPs) in poultry production has been applied for decades worldwide, but recently AGPs have been banned due to the negative consequences for health and food safety. Soybean oligosaccharide (SOS) derived from soybean meal extract is one of natural compound without carrying-over the residue to product and is consumer’s health friendly. The purpose of the present study was to evaluate dietary inclusion of SOS on broiler meat quality. A total of 120 broilers of 7-day-old were allocated into 3 treatments with 4 replications (10 birds each) in completely randomized design. Treatments applied were D1: diet without SOS, D2: D1 plus 0.15% SOS, and D3: D1 plus 0.30% SOS. Intestinal lactic acid bacteria (LAB), protein digestibility, meat protein and fat depositions, and meat cholesterol were the parameters observed. Data were statistically tested using analysis of variance and Duncan test. Dietary SOS inclusion at 0.30% (D3) significantly (P<0.05) increased LAB population (7.21x104 cfu/g), protein digestibility (72.80%), and meat protein deposition (90.83 g/bird), but it decreased meat fat (8.27 g/bird) and meat cholesterol (37.28 mg/100 g). In conclusion, dietary SOS inclusion at 0.30% improves meat quality of broiler based on the increase in meat protein deposition with lower fat and cholesterol.

Keywords: soybean oligosaccharide, meat protein and fat, meat cholesterol, broiler

1. Introduction
Increasing feed efficiency in meat production is an important key for economical broiler industry. The improvement of feed efficiency for economical meat production recently should be a serious consideration compared to the previous decade. The use of antibiotic growth promoters (AGPs) have dramatically increased for the last few decades with the purposes of improving efficiency of poultry production [1]. It has been reported previously that a large amount of AGPs have long been used by the farmers as growth promoter and disease control [1, 2, 3]. In many countries, dietary inclusion of antibiotic growth promoters or AGPs in poultry has not been recommended or has been banned due to the concerns in relation to the development of resistant pathogenic bacteria and gain residue in animal product. The ban on AGPs has prompted nutritionists to investigate an alternative substances beneficial for improving the production performance of poultry. Using natural compound is one possible alternative to feeding antibiotic which is able to improve productivity without producing negative effects on either the host or consumer health. Intestinal health and performance improvements by providing feed without antibiotic growth promoters are the possible way to produce chicken’s clean product.
It was reported that there are over 600 species of bacteria found in the chicken gastrointestinal and their development affected intestinal morphology and function [4]. Shorter villi growth is the indication of the change in intestinal morphology due to the effect of toxin produced by photogenic bacteria and leads the reduction in nutrients absorption and decreased productivity. An alternative feed compound known as prebiotic(s) have natural characteristic without carrying-over the residue to product and consumer’s health friendly. Prebiotic is a nondigestible feed compound that can be fermented by selective bacteria of the host and stimulates growth as well as activity of beneficial bacteria, and on the other hand, depresses harmful bacteria counts, thus improves intestinal health and performance of poultry. Prebiotics are commonly derived from plant sources and one of them can be obtained from soybean meal extract called soybean oligosaccharide (SOS). Some oligosaccharides are known to have function as prebiotic because they are not hydrolyzed by intestinal host enzyme but are able to favorably alter the gut microflora. Soybean oligosaccharide (SOS) could be fermented by selective beneficial bacteria such as Bifidobacteria and Lactobacillus and resulting short chain fatty acid (SCFA) that modulate the intestinal milieu to be healthy condition with lower pH. For example, greater SCFA production has been found in turkey [5], and in broiler [6] fed oligosaccharides associated-soybean meal. Better fermentability of functional oligosaccharide indentified by the SCFA or butyrate production was found with in vitro study [7].

The change in gut bacteria to be stable enhances the animal’s resistance to gastrointestinal infections and provides a positive impact on intestinal wall function [8]. This phenomenon leads to the increase in nutrients digestibility and absorption since the main nutrients utilization is started in the gut due to improved villi growth. Intestinal health indicated by higher villi growth and supported by the increased beneficial bacteria counts, such as lactic acid bacteria (LAB), are the two advantages for the host caused by the additional effect of prebiotic. First, the increased LAB population the higher bile salt hydrolase (BSH) could be produced, and this enzyme has an ability to bind bile salt to be deconjugated form so that diminish the emulsion of lipid fraction and resulted the decreased fat absorption [9], and lowered cholesterol [10]. Second, growth improvement of villi could facilitate the increase in nutrients absorption, especially protein, since these two nutrients are absorbed at low pH condition. It has been described in the previous paragraph that feeding SOS bring about the lower intestinal pH due to the intermedier substances produced by LAB and support absorption. The first phenomenon is supported by the previous study in crossbred local chickens that prebiotic inulin of dahlia tuber at higher level of either powder (1.2%) or extract (1.17%) forms significantly decreased meat cholesterol compared to control (5.02 vs. 9.06 mg/dl) and meat fat (1.66 vs. 4.57%) [11]. Similarly, further study was consistence with the second phenomenon that of prebiotic inulin of dahlia tuber (1.2%) combined with single probiotic Lactobacillus sp. (1.2 mL equal to 10^6cfu/mL) increased meat protein deposition (74.48 vs. 62.731 g/bird) with the same meat Ca mass [12]. Therefore, according to the background as described previously, the effect of dietary supplementation of soybean oligosaccharide (SOS) derived from extract of soybean meal on broiler meat quality based on meat protein deposition and cholesterol content was evaluated.

2. Materials and Methods
2.1. Experimental Animal and Diet
Two hundred and eighty (280) birds of 7-day old broiler with average body weight of 112.2 ± 6.7 g were used in the present study. Experimental diet was composed of corn, rice bran, soybean meal, fish meal, CaCO_3, minerals and vitamins mixture, and soybean meal extract as a source of soybean oligosaccharide (SOS). Extract of soybean meal was insidently prepared at the laboratory using methanol and water as a dilution with a ratio of 50 and 50. Protein and energy contents of the experimental diets for starter period was 22.8% and 2.93 kcal/kg, respectively, and those for finisher period was 20.9% and 2.95 kcal/kg, respectively (Table 1). The birds were provided dietary treatments for 5 wk starting on day 7 until day 42. Feed and drinking water were available ad libitum.
2.2. Parameter and Experimental Design
The experimental birds were distributed into 3 (three) treatments with 4 replications (10 birds each) in a completely randomized design. Dietary treatments were D1: ration without SOS extract, D2: D1 plus 0.15% SOS extract, D3: D1 plus 0.30% SOS extract. Intestinal lactic acid bacteria (LAB), protein digestibility, meat protein and fat mass or depositions, and meat cholesterol were the parameters measured. Data were statistically tested based on analysis of variance and continued to Duncan test at 5% probability level [13].

2.3. Sample Analysis
Intestinal LAB counts in the totally collected digesta from all segments were performed using media deman rogosa sharpe (MRS) combined with eosin methylene blue agar (EMBA). Total plate count method [14] was applied to calculate colony of LAB (cfu/g) with the formula as follows: total colony multiplied by 1 per dilution factor per plate. Protein and fat mass were obtained by multiplying protein and fat percentage of meat, respectively, with the weight of all body meat (15). Determination of meat cholesterol contents were performed according to Leibermann and Burchard method using UV-visible spectrophotometer at a wavelength of 680 nm with slight modification.

Table 1. Experimental diet composition and its nutrient content*.

| Ingredient            | Starter (% ) | Finisher |
|-----------------------|--------------|----------|
| Yellow corn           | 51.50        | 56.00    |
| Rice bran             | 15.50        | 14.80    |
| Fish meal             | 9.00         | 6.50     |
| Soybean meal          | 23.30        | 21.50    |
| CaCO3                 | 0.40         | 0.70     |
| Vitamin-mineral mix   | 0.30         | 0.50     |
| Total                 | 100.00       | 100.00   |

| Nutritional content (%) | Start | Finish |
|-------------------------|-------|--------|
| Metabolizable energy (kcal/kg) | 2932.97 | 2950.25 |
| Crude protein           | 22.85 | 20.89  |
| Ether extract           | 6.38  | 6.02   |
| Crude fiber             | 6.56  | 6.43   |
| Mthionine               | 0.42  | 0.37   |
| Lysine                  | 1.23  | 1.07   |
| Arginine                | 1.38  | 1.24   |
| Calcium total           | 1.04  | 0.96   |
| Phosphorus total        | 0.65  | 0.59   |

*Chemical value of Laboratory analysis results

3. Results and Discussion
Lactic acid bacteria (LAB) population, protein digestibility, and meat protein mass or deposition significantly (P<0.05) increased due to dietary inclusion of higher level (0.30%/D3) of soybean meal extract as soybean oligosaccharide (SOS) source (Table 2). However, dietary addition of soybean meal extract as source of SOS at the levels of either 0.15% (D2) or 0.30% (D3) significantly (P<0.05) decreased meat fat deposition and meat cholesterol content as compared to control (D1). In case of both protein digestibility as well as meat protein deposition indicated the medium values due to feeding additional soybean meal extract at the level of 0.15% (D2), and no significantly different compared to D1 and D3. It has been known that prebiotic function as “food source” that could be fermented by the beneficial endogenous bacteria of the host such as lactic acid bacteria (LAB).
3.1. Intestinal Lactic Acid Bacteria (LAB) Population

The obvious result was found in D3 treatment (Table 2) that dietary inclusion of higher SOS significantly increased LAB population. Some oligosaccharides including SOS, especially in poultry, could be fermented by LAB to produce short chain fatty acids (SCFAs), namely acetate, butyrate and propionate. These fermented products brought about the intestinal milieu to be lower pH which was favourable for the growth of LAB in particular [16], and lead to the increased their population. In addition, SCFAs as the end products of fermentation of dietary SOS, were known to have beneficial effect on energy metabolism in mammalian [17]. Soybean oligosaccharide (SOS) have been reported to be effectively fermented by intestinal Lactobacillus and Bifidobacteria to produce SCFAs and predicted to have lowering-effect on intestinal pH which condition facilitates the growth improvement of these beneficial bacteria, in one side, and inhibits the harmful or pathogenic bacteria, in other side.

Table 2. Intestinal lactic acid bacteria (LAB) population, protein digestibility, and meat quality of broiler fed soybean oligosaccharide derived from soybean meal extract.

| Parameter                        | Soybean meal extract inclusion |
|----------------------------------|-------------------------------|
|                                  | D1 (none) | D2 (0.15%) | D3 (0.30%) |
| LAB population (10^9 cfu/g)      | 4.85 b     | 5.75 b      | 7.21 a      |
| Protein digestibility (%)        | 62.92 b    | 68.59 ab    | 72.80 a     |
| Meat protein deposition (g/bird) | 83.72 b    | 87.10 ab    | 90.83 a     |
| Meat fat deposition (g/bird)     | 11.25 a    | 8.27 b      | 8.49 b      |
| Meat cholesterol (mg/100 g)      | 42.17 a    | 37.28 b     | 36.74 b     |

a,b: Mean value within the same raw followed by different superscript differ significantly (P<0.05).

3.2. Protein Digestibility and Meat Protein Deposition

Phenomenons of either the increased LAB population or the inhibition growth of pathogenic bacteria lead to the improved intestinal health and activity of digestive enzymes of the host animal. Intestinal health indicated by the improved growth of villi is closely related to the better nutrients digestibility, especially protein. As a comparison, feeding a combination of oligosaccharide derived from chicory and Enterococcus faecium increased villus height (774 vs. 614 μm) and villus height/crypt depth (7.13 vs. 4.86) [18]. Thus, it could be concluded that dietary inclusion of a symbiotic oligosaccharide and Enterococcus faecium improved intestinal morphology and nutrient absorption and lead to the increased growth performance. The present study was supported by the previous result [18] that dietary inclusion of SOS at the level of 0.30% (D3) significantly improved protein digestibility (Table 2). Protein digestibility improvement due to stimulating effect of feeding SOS ensures the higher availability of protein supply as a substrate for protein deposition. Stimulating effect of SOS is similar to that of MOS on intestinal development and health in supporting the improvement of nutrients digestibility for better performance in poultry, especially meat protein deposition. Intestinal development could be indicated by the growth of villi in term of height and crypt depth [18,19]. Although these two parameters, villus height and crypt depth, were not determined in present study, the results indicated that protein digestibility was consistent with the improvement of protein deposited into meat due to incusion of SOS at 0.30% in the diet (Table 2). The present study agreed with the previous finding [19] that feeding mannose oligosaccharide (MOS) increased growth of intestinal villi, indicated by the villi height, and followed by numerically increasing nutrients digestibility (protein and starch) in broilers. Villi growth would be a factor stimulates the improvement of nutrients digestibility, especially protein, and finally increase protein supply for meat protein deposition.
3.3. **Meat Fat Deposition and Cholesterol Content**

The decreased meat fat deposition in can be correlated with the activity of gut bacteria as affected by feeding dietary inclusion of SOS at 0.30% (D3). The present study was similar to the previous results [20] that the increased LAB was found in crossbred native chickens fed probiotic inulin derived from dahlia tuber. The present results of either fat meat mass or cholesterol concentration were decreased by dietary addition of SOS was greatly possible to be due to the increasing activity of bile salt hydrolase (BSH), an enzyme produced by lactic acid bacteria (LAB) [11]. The biochemical mechanism of reduced cholesterol in particular, will be discussed in the following paragraph. Short chain fatty acids (SCFAs), product of fermentative effect of intestinal bacteria on SOS was assumed to have important contribution to the decrease in meat fat deposition. This is supported by some studies [21, 22] that SCFAs production derived from the fermentation of prebiotic inulin was due to the high activity of endogenous intestinal bacteria. One component of SCFAs, namely propionate, has an important key in controlling and lowering hepatic lipogenesis and leads to an inhibition of lipogenesis which is related to the decrease in meat fat mass. The enhanced LAB growth is assumed to be correlated with a higher bile salt hydrolase (BSH) that can be produced. BSH enzyme has been reported to exert its effect on the change in bile salt activity to be deconjugated form so that diminish the emulsion of lipid fraction and resulted the decreased fat absorption [9].

Metabolic function and activity of BSH related to either animal or intestinal bacteria could be connected with a given prebiotic such as SOS that was investigated in the present study. It has been described in the previous paragraph that dietary inclusion of SOS at the level of 0.15% (D2) as well as at 0.30% (D3) significantly (P<0.05) decreased meat cholesterol content (Table 2). The increased intestinal LAB population, due to feeding additional SOS, is assumed to be more BSH production and activity causing the depressed cholesterol absorption and resulted lower deposition in the meat. BSH production derived from the endogenous bacteria (LAB) and its activity were the assemble of probiotic strain which could explain cholesterol-lowering mechanism [10]. Previously discussed that gut BSH produced by LAB could change bile salt into deconjugated form [23, 24] which enzyme can not be completely able to hydrolise dietary lipid. This phenomenon have been reported [25, 26] that the decreased fat meat mass and cholesterol content were found in growing chickens fed a combination of prebiotic inulin and single probiotic Lactobacillus sp. The increasing number of lactic acid bacteria (LAB), due to the feeding prebiotic SOS, the higher production and activity of BSH enzyme that could be correlated with meat cholesterol reduction. In relation to the mechanism of fat digestion and absorption, feeding whatever dietary prebiotics including SOS was antagonistic with the usage of antibiotic growth promotors (AGPs). In case of chicken studies demonstrated that inclusion of AGPs in the diet significantly decreased Lactobacillus sp. population, the major intestinal BSH-producer [27]. Therefore, in the absence of using AGPs, BSH is a promising microbial enzyme targeting for the improvement of animal production quality with special reference to meat fat deposition and meat cholesterol content.

4. **Conclusion**

Meat quality improvement based on high protein deposition, low fat mass, and cholesterol content, is found in broiler fed dietary inclusion of soybean oligosaccharide (SOS) derived from soybean meal extract at the level of 0.30% (D3).

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