Feeding reduced dietary protein added with prebiotics on protein metabolism characteristics and growth performance of broiler chickens

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Abstract. The purpose of the present study was to evaluate the effect of feeding prebiotics on protein metabolism characteristics and growth performance of broilers. A completely randomized design with 4 treatments and 5 replications was arranged. Heterophil/lymphocyte (H/L) ratio, protein metabolism characteristics (protein digestibility, fecal N–MH excretion, and meat protein mass), and growth performances (feed consumption, BWG, and FCR) were the parameters observed. Analysis of variance was performed to statistically evaluate the data, and continued to Duncan test at 5% probability. Protein digestibility, and meat protein mass in T1 and T2 were significantly (P<0.05), while those in T0 were significantly (P<0.05). On the contrary, H/L ratio, feed consumption, and FCR were significantly (P<0.05) lowest in both T1 and T2, but those in T0 were significantly (P<0.05). In conclusion, feeding inulin and soy-oligosaccharide extracts improves protein metabolism and it is characterized by the increase in protein digestibility and meat protein mass, and promote better growth of broiler.

1. Introduction
The decrease in dietary protein level in poultry whilst performance should be maintained stable is recently considered as one strategy to reduce feed cost in poultry production. The major cost in poultry production is particularly related to protein sources ingredients composed feed. Broiler chickens require high level of dietary protein because lack of this nutrient supply severely influences their performance. Broilers in particular, need adequate dietary protein supply to support their high growth rate. Formulation of high protein diet is usually composed of protein sources such as fish meal and soybean meal which ingredients are high in price. Feeding suboptimal diet with low protein level through the decreased usage of protein source ingredients is possible to reduce feed cost. In recent year, high nitrogen excretion in relation to environmental problems as well as high price of protein source ingredients have caused the increasing interest in using low protein diets for poultry production [1] [2]. However, dietary protein levels either quantity or quality with its amino acids availability and balance are the limiting factors that can profoundly affect bird performance. It is well documented that low protein supply due to reduced protein diet would be related to the consequence of growth retardation. Increasing utilization efficiency of dietary protein in relation to the setting and
usage of protein source ingredients can be pursued by addition of prebiotics to maintain nutrient absorption, especially protein, at least still sufficient and meet the requirement for poultry production.

It has been previously reported that reducing protein diet up to 10% lower than NRC recommendation with inclusion of either prebiotic or probiotic had no negative effect on growth performance but increased lactic acid bacteria and decreased Escherichia coli counts [3]. In contrast, additional phyogenic compound of essential oil derived from origano and citrus combined with fructo-oligosaccharide was able to overcome the negative effect of a suboptimal diet with both lower energy and protein contents [4]. It seems there are various results concerning the response of the birds given suboptimal diet with inclusion of additive. The different response may depend on additive level and source, preparation method, feeding management, and the strain or age of the birds in relation to feeding dietary protein levels. For example, inclusion of either organic acid, prebiotic or probiotic could improved growth performance of broilers fed diet with 25% protein lower than NRC recommendation [5]. In case of birds maintained in the tropical region, the study results indicated that feeding low protein diet (18%) with inclusion of inulin extract of dahlia (Dahlia variabilis) tuber improved intestinal health, protein digestibility, and increased final body weight and carcass weight in local chickens selected for high body weight [6]. The use of low protein diets with inclusion of prebiotic may be practically useful since this additive play a positive effects on protein utilization which supports the production performance of poultry. Therefore, the study was conducted to evaluate the effects of dietary inclusion of different kinds of prebiotics on protein metabolism characteristics in relation to growth performance of broiler.

2. Materials and Methods

2.1. Animal and Feed
A total of 200 broilers of 2-week old with initial body weight of 475.8 ± 48.4 g were devided into 4 treatments with 6 replications of 8 birds each, and another 8 birds of untreated broiler were plotted for endogenous correction. Dietary treatments were adjusted based on the respective levels of prebiotics namely, inulin of dahlia tuber extract, soy-oligosaccharide of soybean extract, and glucomannan of porang tuber extract. The birds were provided free access of drinking water. A small amount of feed (approximately 30 g) was mixed with prebiotics according to their respective levels. In order to be completely consumed, the mixed feed with prebiotics was given in the morning before feeding other portion of feed without any supplementation to fulfill daily feed requirement. Experimental diets (Table 1) after adding prebiotics according to the level required as treatments were provided from day 15 until 35-day old.

2.2. Parameter Determination
Heterophil-lymphocyte ratio (H/L ratio), protein digestibility, fecal Nτ–methylhistidine (Nτ–MH) excretion, muscle protein deposition (MPD), feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were the parameters measured. H/L ratio was determined based on the number of granular (heterophile, eosinophil, and basophiles) and nongranular (lymphocytes and monocytes) in one hundred leucocytes [7] as previously reported [8]. Analysis method and calculated formula were the same as reported elsewhere for fecal Nτ–MH excretion measurement [9], and for MPD determination [10].

2.3. Experimental Design and Statistical Analysis
The birds were distributed into 4 group of treatments with 6 replications in a completely randomized design. Treatments tested were diet with reduced protein levels and added with prebiotics as follows: R0: 21% protein diet (control), R1: 18% protein diet + 1.2% inulin extract, R2: 18% protein diet + 0.3% soy-oligosaccharide extract, and R3: 18% protein diet + 0.3% glucomannan extract. Analysis of variance was performed to evaluate treatments effect, and continued to Duncan test to differentiate among means value at 5% probability [11] when treatment indicated significant effect.
Table 1. Composition of experimental feed and nutrients content

| Feed Ingredient        | Dietary Treatment | R0   | R1*  | R2*  | R3*  |
|------------------------|-------------------|------|------|------|------|
| Yellow corn            |                   | 48   | 50.3 | 50.3 | 50.3 |
| Rice bran              |                   | 14   | 20   | 20   | 20   |
| Soybean meal           |                   | 27   | 21.2 | 21.2 | 21.2 |
| Fish meal              |                   | 10   | 7.5  | 7.5  | 7.5  |
| CaCO3                  |                   | 0.5  | 0.5  | 0.5  | 0.5  |
| Vitamin and mineral    |                   | 0.5  | 0.5  | 0.5  | 0.5  |
| Dahlia inulin extract  |                   | –    | 1.2  | –    | –    |
| Soy-oligosaccharide    |                   | –    | 0.3  | –    | –    |
| Glucomannan            |                   | –    | –    | –    | 0.3  |
| Total                  |                   | 100  | 101.2| 100.3| 100.3|

Nutrient Content**(%)

|                        | R0   | R1*  | R2*  | R3*  |
|------------------------|------|------|------|------|
| Metabolizable energy***(kcal/kg) | 2978.41 | 2927.86 | 2944.12 | 2950.34 |
| Crude protein          | 21.29| 17.98| 18.21| 18.16|
| Ether extract          | 3.81 | 3.77 | 4.21 | 4.04 |
| Crude fiber            | 4.27 | 4.16 | 4.22 | 4.71 |
| Calcium                | 1.11 | 0.90 | 1.00 | 0.97 |
| Phosphorus             | 0.68 | 0.62 | 0.65 | 0.60 |
| Methionine             | 0.55 | 0.49 | 0.50 | 0.47 |
| Lysine                 | 1.31 | 1.12 | 1.33 | 1.11 |
| Arginine               | 1.53 | 1.31 | 1.49 | 1.28 |

*Nutrients content have been adjusted to 100% unit of total feed
**Determined value of laboratory analysis
***Values were calculated based on the same formula as reported elsewhere [12]

3. Results and Discussion

3.1. Protein Metabolism Characteristic

Protein digestibility and meat protein mass increased to be the higher values due to feeding 18% protein diet with inclusion of 1.2% inulin extract (R1) and that with addition 0.3% soy-oligosaccharide (R2) as compared to R0 (Table 2). However, fecal Nτ–MH excretion did not affected by the treatments although reduced protein diets were given with prebiotics supplementation. The increased protein digestibility in the group receiving additional additives, especially inclusion of inulin. This was due to improvement of intestinal health caused by the fermentation effect of beneficial endogenous bacteria, such as lactic acid bacteria (LAB), that produced short chain fatty acid/SCFA [13]. Production of SCFA was attributable to the reduced intestinal pH that brought about the decreased growth of pathogenic bacteria such as coliform while stimulated the increase in LAB population. The better balance of intestinal bacteria can be an indication of gut health improvement and provided a positive impact on nutrient digestibility, particularly protein, as found in the current study. The present results were similar to the improved protein digestibility as previously found which was supported by the increased LAB population due to feeding soybean oligosachcharide [10], and by the decreased Escherichia coli counts because of diet fortified with dahlia inulin [14].
Table 2. Protein metabolism characteristic in broiler fed reduced dietary protein with prebiotics inclusion

| Parameter                        | Treatment          |
|----------------------------------|--------------------|
| Protein digestibility, %         | T<sub>0</sub>      |
|                                  | 74.12 b            |
|                                  | T<sub>1</sub>      |
|                                  | 80.22 a            |
|                                  | T<sub>2</sub>      |
|                                  | 79.88 a            |
|                                  | T<sub>3</sub>      |
|                                  | 78.04 ab           |
| Fecal N<sub>τ</sub>–methylhistidine (N<sub>τ</sub>–MH), µmol/d | T<sub>0</sub>      |
|                                  | 11.19              |
|                                  | T<sub>1</sub>      |
|                                  | 10.88              |
|                                  | T<sub>2</sub>      |
|                                  | 10.79              |
|                                  | T<sub>3</sub>      |
|                                  | 11.03              |
| Meat protein mass, g/bird        | T<sub>0</sub>      |
|                                  | 110.62 b           |
|                                  | T<sub>1</sub>      |
|                                  | 119.60 a           |
|                                  | T<sub>2</sub>      |
|                                  | 120.15 a           |
|                                  | T<sub>3</sub>      |
|                                  | 114.90 ab          |

<sup>a</sup>–<sup>b</sup> Value in each parameter with different superscript differ significantly (P<0.05)

Protein digestibility did not impact on fecal N<sub>τ</sub>–MH excretion which was proved by the statistically same value, although numerically there was a tendency of decreasing excretion levels. However, protein digestibility, a factor indicating protein and/or amino acids supply, was important for growth with special emphasis on meat protein mass. It was clear that the improved protein digestibility was followed by the increased meat protein mass (Table 2), since fecal N<sub>τ</sub>–MH excretion tended to decrease in T<sub>1</sub> and T<sub>2</sub>. Fecal N<sub>τ</sub>–MH was an intermediate product of amino acid metabolism that can’t be reutilized by the body, so it can be used as an index of protein utilization or muscle protein synthesis [9]. Therefore, it was very logical that the improved meat protein deposition in T<sub>1</sub> and T<sub>2</sub> was due to the effective prebiotic effects to induce the increase in protein supply via protein digestibility as a substrat for body protein synthesis.

3.1. Growth Performance

Feeding reduced protein diet (18%) added with 1.2% dahlia inulin extract (T1) and 0.3% soy-oligosaccharide (T<sub>2</sub>) significantly decreased H/L ratio, feed consumption, and FCR, but BWG in those of both treatments was not different (Table 3). Lower H/L ratio was an indication of higher body resistance due to feeding effects of prebiotics (T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>), although with reduced feed consumption. An interesting phenomenon was that protein digestibility increased (Table 2) although feed consumption decreased (Table 3) in birds fed reduced protein diet added with prebiotics (T<sub>1</sub> and T<sub>2</sub>). The unchanged BWG in T<sub>1</sub> and T<sub>2</sub> treatments did not determine the low values of FCR, but the decreased feed consumption was a principle determinant. Although feed consumption reduced in T<sub>1</sub> and T<sub>2</sub>, body weight gain was not affected (Table 3) since protein digestibility is assumed to have overcoming-effect on protein utilization. It was consistent with study concerning supplementation of phyogenic compound (essential oil) derived from origano or citrus combined with fructo-oligosaccharide that was able to overcome the negative effect of suboptimal diet with lower energy and protein contents [4].

Table 3. Growth performance of broiler fed reduced protein diet with prebiotics inclusion

| Parameter                        | Treatment          |
|----------------------------------|--------------------|
| H/L ratio                        | T<sub>0</sub>      |
|                                  | 0.69 a             |
|                                  | T<sub>1</sub>      |
|                                  | 0.40 c             |
|                                  | T<sub>2</sub>      |
|                                  | 0.42 c             |
|                                  | T<sub>3</sub>      |
|                                  | 0.55 b             |
| Feed consumption, g/bird         | T<sub>0</sub>      |
|                                  | 2196.70 a          |
|                                  | T<sub>1</sub>      |
|                                  | 2075.90 b          |
|                                  | T<sub>2</sub>      |
|                                  | 2069.50 b          |
|                                  | T<sub>3</sub>      |
|                                  | 2172.00 a          |
| Body weight gain (BWG), g/bird   | T<sub>0</sub>      |
|                                  | 1234.10            |
|                                  | T<sub>1</sub>      |
|                                  | 1305.60            |
|                                  | T<sub>2</sub>      |
|                                  | 1309.80            |
|                                  | T<sub>3</sub>      |
|                                  | 1270.20            |
| Feed conversion ratio (FCR)      | T<sub>0</sub>      |
|                                  | 1.78 a             |
|                                  | T<sub>1</sub>      |
|                                  | 1.59 b             |
|                                  | T<sub>2</sub>      |
|                                  | 1.58 b             |
|                                  | T<sub>3</sub>      |
|                                  | 1.71 a             |

<sup>a</sup>–<sup>c</sup> Value in each parameter with different superscript differ significantly (P<0.05)
Dietary inclusion of inulin extract and soy-oligosaccharide seem to be more effective to increase nutrient efficiency, protein in particular, through the improvement of intestinal health as described in the previous paragraph. Previous study indicated that additional of commercial prebiotic (fermacto) to diets containing protein 10% lower than NRC recommendation. It had same effect compared to control diet on performance of broilers [15]. Feeding additives in general and prebiotics in particular would be beneficial for poultry. It given suboptimal diets with low protein level. Previous study proved the fact that low protein diet (18%) added with inulin extract of dahlia (Dahlia variabilis) tuber stimulated villi height, improved protein digestibility, and increased final body weight and carcass weight in crossbred local chickens [6]. The effects of dietary inclusion of glucomannan extract was different from that of inulin extract supplementation. Results of the present study indicated that inclusion of 0.3% glucomannan extract (T1) to low protein diet reduced H/L ratio, but feed consumption and FCR were found to be as high as those of control group (21% protein). Level and type of prebiotics, natural or commercial sources, may determine the response of the birds to the protein level of feed.

A commercial prebiotic of mannan-oligosaccharide at 0.1% exhibited positive effect on body weight and FCR in broiler given feed 10% protein below NRC recommendation, as compared to the birds fed the same feed without any additive supplementation [16]. However, when supplementation of glucomannan extract at 0.1 to 0.2% to feed of normal protein level improved protein digestibility, increased muscle protein mass and body weight gain [17]. The decreased H/L ratio and coliform counts, and the increased LAB population with the same levels of glucomannan extract were also previously observed [18]. Therefore, dietary inclusion of additives depends on the levels of protein diet. It shows that when low protein diet is fed to broiler chicken, higher level of additives is needed.

4. Conclusion

Inulin and soy-oligosaccharide extracts was added to reduced level of protein diet in order to improve protein metabolism. It was characterized by the increase in protein digestibility and meat protein mass, and promote better growth of broiler.

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