Healthy meat production of broiler fed microparticle-protein diet with inclusion of inulin derived from dahlia tuber extract

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Abstract. The present study was conducted to evaluate the healthy edible meat characteristic of broiler due to feeding effect of microparticle-protein diet with inclusion of inulin from dahlia tuber extract. Fish and soybean meal were exposed to ultrasonic bath to obtain microparticle prior to feeding trial. 160 broilers (2-week old) were allocated into 4 treatment. Treatments were tested as follows T0: 21% intact protein diet, T1: 18% intact protein diet, T2: 18% microparticle-protein diet, T3: diet T2 + 1.2% dahlia inulin extract. A completely randomized design with 4 treatments and 5 replications (8 birds each) was arranged. Intestinal Escherichia coli, heteroph-lymphocyte (H/L) ratio, meat protein and fat mass, meat cholesterol, and carcass weight were the parameters observed. Analysis of variance was applied, and continued to Duncan test. Results showed that all parameters, except carcass weight, were significantly (P<0.05) affected by dietary treatments. Escherichia coli count, H/L ratio, meat cholesterol, were significantly (P<0.05) decreased by T2 and T3 treatments. Meat fat mass was reduced by T3 treatment, but carcass weight was not affected by any treatments.

In conclusion, diet composed of 18% microparticle-protein with inclusion of 1.2% inulin extract produces healthy edible meat of broiler characterized by lower meat fat mass and cholesterol with higher meat protein mass.

Keywords: microparticle protein, inulin dahlia tuber, meat protein mass, meat cholesterol, broiler

1. Introduction

Fish meal and soybean meal are the main protein sources for poultry feed component that should be available to meet the requirement of the birds. The adequacy of protein and/or amino acids is usually obtained from the feed composed of high portion of protein source. The high price of feed can be confirmed due to the use of high portion of fish meal and soybean meal. Feed price can be reduced by minimalizing the use of protein source component, but it has to be anticipated the possibility of low protein supply that can impair productivity. Processing protein source ingredients to become microparticle is one possible alternative way to increase or at least to maintain protein utilization when the bird was given low content of dietary protein. Previous studies indicated that the increased protein digestibility, nitrogen retention, and some essential amino acids digestibility [1] was found in broiler force fed with microparticle protein of fish meal and soybean meal. Particle size of feed was known to have several advantages on the aspects related to poultry productivity such as gastrointestinal tract development, nutrients utilization, and productive performance [2,3,4]. It has been reported that particle size reduction was correlated with the increase in the number of particles as well as the surface area per unit volume and allowing greater access to digestive enzymes [5,6]. Therefore, it is due to an
intensive activity of digestive enzyme to the smaller feed particle size allows the increase in nutrients, especially protein, absorption.

Research regarding particle size of feed ingredients for poultry in general were available elsewhere [3,4], but the information about feeding microparticle protein was very limited. Feeding microparticle protein diet is suggested to be much more efficient when combined with natural additive such as prebiotic. Feeding diet with non-antibiotic additives inclusion have been increasingly important to improve poultry production, especially edible meat quality of broiler. Since dietary inclusion of antibiotic growth promoters in poultry has been banned due to the awareness of the importance of consumer health and of nutritional value of animal product, thus the research was focussed on the use of natural feed additive. Natural additive that can be classified as probiotic is known as inulin which has been reported by [7]. Prebiotics inulin can be supplemented into the poultry feed without antibiotic growth factor due to its role in modulating the body resistance improvement of host animal, and in producing healthy meat for consumer [8]. Inulin, among other types of prebiotic, is a prebiotic derived from plants with a significant effect on the chicken productivity. Tuber plant called Dahlia variabilis is a potential source of inulin in Indonesia. The present study was conducted to evaluate the effect of feeding microparticle protein combined with inulin extract of dahlia tuber on healthy edible meat of broiler chicken.

2. Materials and Methods

2.1. Experimental Animal and Feed

Protein source ingredients, fish meal and soybean meal, were firstly ground to reduce particle size. Finely ground ingredients were dissolved in distilled water and added with virgin coconut oil to prepare stable dispersion of solution prior to performing on ultrasound transducer [5]. The animals used in the present experiment were 160 birds of 2-week old broiler with initial body weight of 463.8 ± 50.6 g, and the birds were devided into 4 dietary treatments. The birds were given free access of drinking water, but feeding dietary treatment was adjusted based on inulin level. Inulin extract was mixed in a small amount (approximately 25 g) of feed and was given in the morning to ensure it can be completely consumed. The remaining feed without inulin for daily need was provided thereafter. Feeding dietary treatments (Table 1) were started on day 15 and completed when the birds were 35-day old.

2.2. Experimental Parameter

Parameters observed in the present study were grouped into either the aspects of healthy host animal, indicated by intestinal Escherichia coli count and heterophyl-lymphocyte (H/L) ratio, or the items of the healthy meat production, namely meat protein mass, meat fat mass, meat cholesterol, and carcass weight. Total Escherichia coli was measured from digesta of 2 dissected birds of respective replication after collecting blood sample for H/L ratio determination. Nongranular leucocytes (lymphocytes and monocytes) were counted as the basic heterophile-lymphocyte ratio calcultaion [9]. Leibermann – Burchard reaction was used to determine meat cholesterol concentration using spectrophotometer at a wavelength of 680 nm. Escherichia coli count determination was performed using total plate count [10] with the following formula: [total colony (cfu/g) = colony x 1/dillution factor) x 1/(sample weight)]. Other parameters namely, meat protein mass, meat fat mass, and meat cholesterol were measured from meat sample of the same birds as used for Escherichia coli and H/L ratio determination after weighing carcass weight. Meat protein and fat mass were calculated based on the formula previously developed [11] as follows: [mass of meat protein or fat = % meat protein or fat content x meat or fat mass (g)].

2.3. Experimental Design and Statistical Analysis

Dietary treatments (Table 1) applied were as follows: T0: 21% intact protein diet (control), T1: 18% intact protein diet, T2: 18% microparticle-protein diet, T3: diet T2 fortified with 1.2% dahlia inulin
A completely randomized design with 4 treatments and 5 replications (8 birds each) was assigned in the present study. Analysis of variance was applied to test the treatments effect, and continued to Duncan test at 5% probability when the treatment indicated significant effect.

Table 1. Composition and nutrients content of experimental feed

| Feed Ingredient       | Dietary Treatment | T0   | T1   | T2   | T3*  |
|-----------------------|-------------------|------|------|------|------|
|                       |                   | (%)  | (%)  | (%)  | (%)  |
| Yellow corn           |                   | 48   | 50,5 | 50,2 | 50,2 |
| Rice bran             |                   | 14   | 20   | 20   | 20   |
| Intact soybean meal   |                   | 27   | 21   | –    | –    |
| Microparticle soybean |                   | –    | –    | 21   | 21   |
| fish meal             |                   | 10   | 7.5  | –    | –    |
| Microparticle fish    |                   | –    | –    | 7.5  | 7.5  |
| meal                  |                   | 0.5  | 0.5  | 0.5  | 0.5  |
| Vitamin and mineral   |                   | 0.5  | 0.5  | 0.5  | 0.5  |
| Dahlia inulin extract |                   | –    | –    | –    | 1.2  |
| Total                 |                   | 100  | 100  | 100  | 101.2|

Nutrient Content***(%)

| Metabolizable energy**(kcal/kg) | T0   | T1   | T2   | T3*  |
|---------------------------------|------|------|------|------|
| 2978,41                         | 2948,32 | 2978,41 | 2913,36 |
| Crude protein                   | 21,29 | 18,12 | 21,29 | 17,91 |
| Ether extract                   | 2,81  | 2,57  | 2,81  | 2,54  |
| Crude fiber                     | 4,27  | 4,77  | 4,27  | 4,71  |
| Calcium                         | 1,03  | 0,88  | 1,03  | 0,87  |
| Phosphorus                      | 0,65  | 0,61  | 0,65  | 0,60  |
| Methionine                      | 0,45  | 0,39  | 0,45  | 0,38  |
| Lysine                          | 1,37  | 1,12  | 1,37  | 1,11  |
| Arginine                        | 1,51  | 1,28  | 1,51  | 1,26  |

*Nutrients content have been adjusted to 100% unit
**Determined value of chemical analysis
***Values were calculated based on the common formula previously applied [12]

3. Results and Discussion

3.1. Host Animal Condition

Total intestinal Escherichia coli and heterophile-lymphocyte ratio (H/L ratio) were defined as the indicator of the health of host animal which then imply to its correlation with the production of healthy edible meat. Escherichia coli (E. coli) count and H/L ratio were significantly (P<0.05) affected by feeding microprotein diet. Treatments of feeding diet with microparticle protein either with or without inulin inclusion decreased both E. coli count and H/L ratio (Table 2). The reduced total intestinal E. coli implied the improvement of animal health with higher body resistance indicated by lower H/L ratio. As it has been reported previously that dietary inclusion of inulin decreased E. coli in crossbred local chickens [13], and increased lactic acid bacteria in broiler fed soybean oligosaccharide [14] due to the fermentative effect of beneficial bacteria in the intestine. It is well documented that low molecule weight carbohydrate derived from feed component or exogenously added prebiotic inulin can be selectively fermented by the presence of intestinal lactic acid bacteria (LAB) in general, to produce short chain fatty acids (SCFA) or lactic acid. The fermented product brought about the intestinal pH reduction and lead to the decreased pathogen growth such E. coli [13,15]. Similarly, the decreased counts of E. coli and increased Lactobacillus population
were found in broiler given feed additive of a blend of commercial prebiotic and probiotic [16]. Therefore, it can be briefly concluded that intestinal microbes balance is beneficially influenced by dietary inclusion of prebiotics, as it has been previously discussed.

A principle scientific phenomenon is clear that prebiotic could improved the health of host animal or with another term it provided a beneficial physiological effect [17]. The balance of gut microflora plays an important role in nutrition utilization and health of host animal. In other side, feeding microparticle protein alone or in combination with inulin, although at low level (18%), provided the higher availability of protein and also amino acids that facilitating the improvement of body resistance. The increased protein and essential amino acids digestibilities in broiler fed single microparticle protein source ingredients which supported the present results have been previously reported [1,18]. It can be assumed that the adequacy of nutritional supply (protein) is the important factor supporting the improvement of organ related to the body resistance. The present result was comparable to the previous study that body resistance of crossbred local chickens improved, indicated by lower H/L ratio, due to feeding intact protein diet added with dahlia inulin [8]. Body resistance improvement found in the present study was indicated by the decrease in the ratio of H/L to be 0.66 and 0.68 in T2 and T3 treatments, respectively, was near to the normal value (0.5). In crossbred native chicken for example, the reduced H/L ratio can be clearly explained by the results of orthogonal polynomial test that almost 60% of the value was affected by dietary inulin extract inclusion.

Table 2. Edible meat quality of broiler given microparticle-protein diet fortified with dahlia inulin extract

| Parameter                   | Dietary Treatment |
|-----------------------------|-------------------|
|                             | T0   | T1     | T2     | T3     |
| Eschericia coli (10^6 cfu/g) | 5.99 b | 6.18 a | 5.12 bc | 4.48 c |
| H/L ratio                   | 0.77 a | 0.80 a | 0.66 b | 0.68 b |
| Meat protein mass (g/bird)  | 109.68 b | 98.09 c | 110.88 b | 120.93 a |
| Meat fat mass (g/bird)      | 55.71 a | 47.02 b | 46.55 b | 38.54 c |
| Meat cholesterol (g/bird)   | 10.12 a | 8.49 ab | 7.55 b | 7.68 b |
| Carcass weight (g/bird)     | 795.4 | 775.8 | 796.2 | 802.2 |

* Mean values within column followed by different superscript are significantly different (P<0.05)

T0: 21% intact protein diet (control); T1: 18% intact protein diet; T2: 18% microparticle-protein diet; T3: diet T2 fortified with 1.2% dahlia inulin extract

3.2. Edible Meat Production Characteristic

Meat protein mass, meat fat mass, meat cholesterol, and carcass weight observed in the present study were categorized as edible meat production, and all were significantly (P<0.05) influenced by the treatment. Animal host condition in term of birds health condition as have been previously discussed definitely influences the grade of product, in this case was the quality of broiler meat. Edible meat quality can be categorized as healthy meat depend on the characteristics of high and low dietary nutrient deposited during the rearing period. In the present study, the quality of healthy edible meat was charaterized by protein, fat and cholesterol that can be retained into the product as meat protein mass, fat protein mass, and meat cholesterol, respectively. Lower dietary microparticle protein (18%) fortified with dahlia inulin extract (T3) was able to significantly (P<0.05) increase meat protein mass, and on the other hand, decreased both meat fat mass and meat cholesterol concentration (Table 2). It can, therefore, be suggested that reduced protein diet didn’t severely affect productive performance of broiler since protein source ingredients were processed to be microparticle prior to feed formulation and feeding. The unchanged carcass weight implied that the final output of productive characteristic was not affected by feeding low dietary protein. Even with intact protein [15] reported that reducing dietary protein by 10% had no any negative effect on broiler production.
The increase in protein and some essential amino acids digestibilities in broiler fed microparticle of single protein source ingredients [1,18] were the important nutritional factor for the improvement of protein deposition in the form of meat protein mass (Table 2). On the other hand, the lowest meat protein mass was found when low dietary intact protein (T1) was fed. This condition was presumably due to the indirect effect of higher counts of intestinal E. coli that can inhibited digestibility and lowered protein absorption as substrate for protein deposition. For this reason, it can be explained by feeding dietary protein microparticle, although at lower level (18%), added with prebiotic inulin were the important determinant in improving healthy edible meat production in broiler. Some previous studies [19,20,21] stated that dietary inclusion of prebiotics improved the performance of broiler chickens.

A positive effect of feeding either prebiotics or probiotics on protein utilization have been previously reported [22] that broiler fed diet containing low levels of protein and Ca had poorer productive performance as compared to that of control group. However, nutrients retention was alleviated by supplementation of prebiotic, and improved its utilization for higher productive performance quality. An interesting result shown that the performance of broiler given low protein diet was improved by prebiotic supplementation [23]. Scientific perception has been set from the viewpoint of beneficial effect of prebiotic inulin inclusion on protein utilization, and the effect was more evident under suboptimal nutritional levels. Therefore, dietary inclusion of inulin eliminate the possible unfavourable effects of low dietary protein, including its relation to the feeding diet in the form of microparticle protein. This phenomenon can be proved by the results of the present study that meat protein deposition (meat protein mass) increased, but on the other hand, meat fat and cholesterol concentration decreased.

Mechanism and factors attributable to the increase in meat protein mass have been previously discussed, but that concerning meat fat deposition is described in the following description. The decreased E. coli counts was associated with the increased lactic acid bacteria (LAB) due to feeding effect of dietary inclusion of inulin [8,13,15], and soybean oligosaccharide [14]. An enzyme called bile salt hydrolase (BSH) produced by LAB bind to bile salt to be deconjugated form and diminished the emulsion of dietary lipid fraction and brought about the decrease in fat absorption [24], and lowered cholesterol [25]. In relation to lowered fat and cholesterol absorptions, the present study showed the fact that broilers provided microparticle protein diet fortified with inulin resulted high quality product with healthy edible meat. Healthy edible meat was characterized by low meat fat mass and cholesterol concentration but high protein deposition (meat protein mass).

4. Conclusion
Healthy edible meat of broiler characterized by high meat protein mass with low meat fat mass and meat cholesterol can be produced by feeding diet with low level microparticle protein (18%) fortified with dahlia inulin extract (1.2%). The increase in edible meat production quality is attributed to the positive impact of healthy host animal improvement.

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