Feeding microparticle protein sources composed-diet with addition of natural additive to produce clean product of broiler for consumer health friendly

N Suthama, B Sukamto, I Mangisah and L Krismiyanto
Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang 50275, Central Java, Indonesia

E-mail: nsuthama@gmail.com

Abstract. Evaluation of feeding diet composed of microparticle protein source added with inulin of chicory root and *Lactobacillus acidophilus* on intestinal bacteria population, protein digestibility, and performance of broiler was the purpose of the present study. Experimental animals were 248 birds of day old broiler with body weight of 48.2±0.50g (240 birds for the main experiment, and 8 birds for endogenous correction). Feed composed of microparticle protein source added with inulin of chicory and *Lactobacillus acidophilus* at 1.2%, respectively. A completely randomized design with five treatments and six replications (8 birds each) was set in the present study. Treatments tested were R0: diet with 20% intact protein (control); R1: diet with 18% microparticle protein, R2: R1 + 1.2% inulin of chicory; R3: R1 + 1.2% *Lactobacillus acidophilus*; and R4: R1 + 1.2% *Lactobacillus acidophilus* + 1.2% inulin of chicory. Total lactic acid bacteria (LAB) and coliform, protein digestibility, and broiler performances (muscle protein mass, feed intake (FI), body weight gain (BWG), and feed conversion (FCR) were the parameters observed. Data were subjected to analysis of variance and continued to Duncan test at 5% probability level. LAB population significantly (P<0.05) increased, and in contrast, coliform count and FCR significantly (P<0.05) decreased in R2, R3 and R4. Protein digestibility and BWG improved in R3 and R4, but FI enhanced only in R4. In conclusion, feed composed of microparticle protein sources with addition of either chicory inulin or *Lactobacillus acidophilus* at 1.2%, respectively, increase total LAB, protein digestibility and growth performances of broilers. The combination of both additives have more profound effect on productivity and produce a healthy meat product indicated by high muscle protein mass derived from host animal with low pathogen.

1. Introduction

Fast growing broiler for meat production at a relatively short period should be supported by high-grade feed with high protein supply. Dietary protein is usually comprised by much more component of protein source ingredients in order to meet requirement. The expensive feed cost is closely related to the level of fish meal and soybean meal used as the common protein sources in poultry feed. Decreasing protein source component in the diet accompanied by processing that can be positively hope to reduce feed cost. The processing of fish meal and soybean meal to become microparticle have been previously reported to have some advantages. Improvement of Nitrogen retention and protein
digestibility were found in broiler force fed single microparticle-processed fish meal or soybean meal [1]. Similarly, broiler given fish and soybean meal microparticle in pelleted form increased protein and amino acids digestibility, and Ca retention. [2]. Also, the same protein source composed-diet added with Lactobacillus sp. [3], and dahlia inulin [4] improved gut health, carcass characteristic, and performance of broiler. The increased productive performance was correlated with the improved gastrointestinal development and nutrients utilization due to the feeding reduced particle size of feed [5,6,7]. Particle size reduction provided an impact on the increase in either the number of particles or the surface area per unit volume and bringing about the greater accessibility of digestive enzymes on substrates [8,9].

There were many studies have been previously conducted concerning particle size effect of feed component or single ingredient on gastrointestinal tract development, nutrients utilization as well as performances in poultry [10,11]. However, none of the feeding diet composed of microprotein source derived from fish meal and soybean meal was conducted. This feeding management is supposed to be more efficient due to the increase in digestibility and possibly relating with the effort of lowering protein sources in the diet. Feeding low dietary protein level in a common case known a suboptimal diet would be effective when added with feed additive. The reduced dietary protein level by 10% had no negative effect on broiler performance, but additives (prebiotic and probiotic) improved intestinal bacterial balance [12]. Addition of prebiotic to low protein diet of 10% lower than NRC recommendation resulted the same performance as compared to control group in 42-day broilers [13].

Omitting antibiotic growth promoters (AGPs) from the feed inclusion have been increasingly important to improve poultry production in case of clean product for consumer health. Since the use of AGPs have been banned, thus, the study of prebiotic and/or probiotic on the change in intestinal microorganism population in relation to productivity have significantly increased. Non-digestible substance called prebiotic cannot be digested by host animal but it can be fermented by selective bacteria and able to modulate activity of beneficial bacteria. Prebiotic, in contrast, depresses pathogenic bacteria counts, and gives an impact on the improvement of intestinal health and performance of poultry. Feeding prebiotic soybean oligosacharide in normal protein diet [14] increased LAB number or addition of inulin [4] to the microparticle protein composed-feed have been known to reduce Escherichia coli population in broiler. Similarly, research concerning inclusion of dahlia inulin alone [15] and in combination with Lactobacillus sp. to normal protein feed have been observed in local chickens [16]. Based on the background described above the present study was conducted to evaluate the effect of adding a mixture of chicory inulin and Lactobacillus sp. on intestinal microbial condition and production performance in broiler fed reduced level of protein diet.

2. Materials and Methods

2.1. Experimental Animal and Feed

The present study was conducted using 250 birds of 7-day broiler (10 birds for endogenous correction) with average body weight of 219.4 ± 21.8 g. Feed was composed of corn, rice bran, soybean meal (intact and microparticle), fish meal (intact and microparticle), CaCO3, premix, lysine and methionine. Chicory inulin extract and Lactobacillus sp. were the additives used for treatment. Experimental feed was one-step formulation with 20 and 18% protein for intact and microparticle diet, respectively, with energy content of 2990 kcal/kg in average. (Table 1). Dietary treatments were served ad libitum for 5 weeks starting on day 7 until day 42, and given free access to drinking water.

2.2. Experimental Design and Statistical Analysis

A completely randomized design with 5 treatments and 6 replications of 8 birds each was arranged in the present study. Dietary treatment applied were: R0: diet with 20% intact protein (control); R1: diet with 18% microparticle protein, R2: R1 + 1.2% inulin of chicory; R3: R1 + 1.2% Lactobacillus acidophilus; and R4: R1 + 1.2% Lactobacillus acidophilus + 1.2% inulin of chicory. Total lactic acid bacteria (LAB) and coliform, protein digestibility, muscle protein mass (MPM), feed intake (FI), body
weight gain (BWG), and feed conversion (FCR) were the parameter tested using analysis of variance and Duncan test (P<0.05).

2.3. Parameter Measurement
Total lactic acid bacteria (LAB) and *Escherichia coli* counts from digesta of all intestinal segments were performed using deMan Rogosa Sharpe (MRS) medium combined with eosin methylene blue agar (EMBA) based on the total plate method [17]. The colony of LAB and *E. coli* (cfu/g) was calculated based on total plate count with the formula of total colony multiplied by 1 per dilution factor per plate. Digestibility trial was performed through the modification of total collection method with indicator of Fe2O3. Meat protein mass was obtained by multiplying meat protein percentage with total weight of meat [18].

### Table 1. Experimental Feed Composition and Nutrition Content

| Ingredient                        | Feed Composition |
|-----------------------------------|------------------|
|                                   | Intact protein   | Microparticle protein |
|                                   | (20 %)           | (18 %)               |
| Yellow corn                       | 55.00            | 56.50                |
| Rice bran                         | 14.40            | 14.00                |
| Intact soybean meal               | 21.50            | –                    |
| Microparticle soybean meal        | –                | 21.20                |
| Intact fish meal                  | 8.00             | –                    |
| Microparticle fish meal           | –                | 7.2                  |
| CaCO3                             | 0.50             | 0.5                  |
| Premix                            | 0.25             | 0.25                 |
| Lysine                            | 0.10             | 0.10                 |
| Methionine                        | 0.25             | 0.25                 |
| TOTAL                             | 100              | 100                  |

| Nutritional content* (%)          | Metabolizable energy** (kcal/kg) | 2,987.2 | 2,991.8 |
| Metabolizable energy** (kcal/kg)  | 20.52            | 18.49                |
| Crude protein                     | 5.23             | 4.81                 |
| Ether extract                     | 6.68             | 5.77                 |
| Crude fiber                       | 1.14             | 1.07                 |
| Phosphorus                        | 0.93             | 0.88                 |

* Determined value according to chemical analysis
**Calculated value based on Bolton [19] formula

3. Results and Discussion
3.1. Bacterial Counts and Protein Digestibility
Dietary addition of inulin (R2) or *Lactobacillus* sp. (R3) alone as well as combination of both (R4) significantly (P<0.05) increased intestinal LAB population, and on the other hand decreased coliform counts (Table 2). However, the increased total LAB was numerically highest, and the decreased coliform populations was the lowest with combination of inulin and *Lactobacillus* sp. Protein digestibility was also indicated similar pattern with those of intestinal bacteria, but only R3 and R4 were significantly (P<0.05) increased. The increased LAB population and the decreased coliform counts are the indication of the improved balance of microbe that brings about the better health of the intestinal tract. Probiotic inulin a source of “nutrition” for the beneficial bacteria can be fermented especially by *Lactobacillus* sp. exogenously added or by endogenous LAB. Low intestinal pH in broilers fed dietary addition of probiotic and prebiotic as reported previously [20] might be due to fermentation products such as either short chain fatty acid (SCFA) or lactic acid. Healthier condition of gastrointestinal tract can provide the positive effect on nutrient digestibility. Better gut health was
associated with the enhanced LAB and higher antioxidant level in broiler given additional *Lactobacillus* sp. combined with ginger and turmeric extracts [21]. The improved protein digestibility (Table 2) found in the present study was a fact that the data can proved the mechanism due to feeding inulin and *Lactobacillus* sp. Results of the present study was consistent with the previous report [22] that gut morphology improved and LAB population tended to increase due to dietary inclusion of either organic acid or prebiotic as well as its combination. The improvement of gut health due to the decrease in coliform population is closely related to the intestinal ecosystem with the better villi growth and possibly causes greater access of digestive enzyme to substrate or feed. This phenomenon of intestinal condition provides concomitant effect with feeding diet composed of microparticle protein sources in relation to the activity of digestive enzymes.

Table 2. Intestinal Bacterial and Protein Digestibility of Broiler Fed Microprotein Composed-
Diet Added with Chicory Inulin and *Lactobacillus acidophilus*

| Parameter                  | Dietary Treatment | R0    | R1    | R2    | R3    | R4    |
|----------------------------|-------------------|-------|-------|-------|-------|-------|
| LAB, 10^8 cfu/g            |                   | 1.28c | 1.55c | 2.78a | 2.80a | 3.24a |
| Coliform, 10^6 cfu/g       |                   | 3.28a | 2.46a | 1.44b | 1.30b | 1.28b |
| Protein digestibility, %   |                   | 78.14b | 78.11b | 79.58b | 83.87a | 86.72a |

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

3.2. Growth Performance

Dietary inclusion of either chicory inulin (R2) or *Lactobacillus acidophilus* (R3) as well as the combination of both (R4) significantly (P<0.05) improved muscle protein mass (MPM), and body weight gain/BWG (Table 3). Feed consumption (FI) was significantly (P<0.05) increased only by feeding a combination of inulin and *Lactobacillus acidophilus* (R4). However, feed conversion ratio (FCR) was significantly reduced due to dietary inclusion effect of inulin or *Lactobacillus acidophilus* alone and their combination. The increased MPM can be correlated with the improved protein digestibility as substrate provider for synthesis process in term of meat or muscle protein mass. It was similar to the previous study that protein digestibility and BWG improved due to LAB population increased with feeding soybean oligosaccharide [14], and the number of *Escherichia coli* reduced because of dietary inclusion of dahlia inulin [4]. Also, the present result was similar to the previous study that dietary addition of phyogenic compound of oregano and fructooligosaccharide could avoid the negative effect of low protein and energy-containing feed [23]. Feeding low protein diet (10% below NRC recommendation) with inclusion of commercial prebiotic (Fermacto) resulted the same performance of broiler to control group [13]. Diet low in protein level categorized as suboptimal feed, thus, the addition of prebiotic and/or probiotic would be economically beneficial for poultry.

Results of the current study indicated that inclusion of a combination of chicory inulin and *Lactobacillus acidophilus* (R4) improved almost all parameters observed, except feed consumption was found to increase (Table 3). However, since BWG increased at much higher level in R4, so the FCR was still lower than that in control group (R0), even FCR in R2 and R3 with addition of chicory inulin and *Lactobacillus acidophilus* alone, respectively, were also significantly lower than R0 and R1 treatments. An experiment conducted previously indicated that the improved villi height brought about the increased protein digestibility and body weight in crossbred local chicken fed low (18%) protein diet [18]. Growth promoting effect of either dietary inclusion of prebiotic or probiotic, and its combination is recently increasing interest for both farmer and consumer due to higher quality of carcass meat production. Reduced protein diet with inclusion of both commercial prebiotic (Fermacto) and acidifier (Biotronic®SE) resulted the improvement of carcass characteristic and low blood cholesterol of broiler chickens at 45-day old [24]. Application of feed composed of microparticle protein sources added with prebiotic and probiotic would be very promising cost-effective for feed and feeding management in poultry.
Table 3. Growth Performance of Broiler Fed Microprotein Composed-Diet Added with Chicory Inulin and Lactobacillus acidophilus

| Parameter                        | Dietary Treatment |
|----------------------------------|-------------------|
|                                  | R0    | R1    | R2    | R3    | R4    |
| Muscle protein mass, g/bird      | 114.30bc | 102.18bc | 118.41bc | 119.91bc | 126.10bc |
| Feed consumption, g/bird         | 2,790b   | 2,814ab  | 2,747b   | 2,747b   | 2,884a   |
| Body weight gain, g/bird         | 1,476b   | 1,458b   | 1,543ab  | 1,588a   | 1,611a   |
| Feed conversion ratio            | 1.89b    | 1.93ab   | 1.78c    | 1.78c    | 1.79c    |

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

4. Conclusion

Dietary inclusion of either chicory inulin or Lactobacillus acidophilus to the feed composed of microparticle protein sources improves intestinal microenvironment and growth performance. The combination of both additives have more profound effect on productivity and produce a healthy meat product indicated by high muscle protein mass derived from host animal with low pathogen.

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