Coconut (*Cocos nucifera*) and Salak (*Salacca zalacca*) polysaccharides in the diets of *Escherichia coli*-challenged broilers

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Abstract. Palm polysaccharides were believed to contain mannose monosaccharides that are beneficial as an antibiotic replacer for poultry. This experiment was conducted to study the effect of coconut and salak polysaccharides on growth performance, excreta dry matter, digestibility, and bursa of Fabricius weight of *E. coli* challenged broilers. A total of 140-day old unsexed broiler Cobb chicks were used as experimental birds for 4 weeks. During the first week, the chicks were allocated in five brooder pens and transferred into 20 pens on day 8. The broiler chicks were offered 5 different experimental rations. The rations were control basal diet (T1), basal diet added with antibiotic avilamycin (T2), basal diet added with commercial yeast oligosaccharides (T3), basal added with coconut polysaccharides (T4), and basal diet added with Salak (*snake fruit*) polysaccharides (T5). To protect the birds from New Castle diseases, vaccination was done on day 3. On day 14, the birds were challenged against *E. coli* for three consecutive days. Plastic feeders and drinkers were put in the pen. The experiment used a completely randomized design with five different rations, four replicate pens of 7 birds each. The data were subjected to the analysis of variance. Any significant differences detected in the variance analysis were then tested with the least significant difference test. Results indicated that after challenged with *E. coli*, birds fed the basal diet had lower body weight gain, mortality, excreta dry matter, and carcass percentage, but higher in FCR and bursa of Fabricius weight than the birds fed T2, T3, T4, and T5 diets. Feed intake of the *E. coli* challenged broilers were lower than those of broilers fed the T3 diet. Feed digestibility was not affected by the treatment diets. In conclusion, the addition of feed additives either antibiotic or palm polysaccharides could enhance the growth performance, carcass percentage, and excreta dry matter of the *E. coli* challenged broilers.

1. Introduction

Studies on polysaccharides, especially mannose-based polysaccharides from the Palmae plants as a source of "nutricine" (nutrition for health) are rarely available in the database. Sundu et al (2006;2009) pioneered studies in this field [1,2]. Since then, several researchers have begun to become interested in studying the benefits of mannose-based polysaccharides for livestock health. Sundu et al (2015) found that mannose-based polysaccharide products provide new hope in playing a role to maintain health status [3]. However,
studies on mannose-based polysaccharides as a source of prebiotic have been merely focused on the use of copra and palm kernel meal products. Study on other Palmae species has been scarcely done concerning the benefits of their polysaccharides for animal health.

From the nutritional aspect, the carbohydrates content of coconut meal and palm kernel meal were mainly mannose-based polysaccharides (mannot). It is expected that other palm nuts might have the same profiles of carbohydrates. As a tropical country, Indonesia produces various species of Palmae nuts such as Salak (Salacca zalacca) and coconut (Cocos nucifera). Those palm nuts might have the same mannose-based polysaccharide as in coconut and oil palm nut and thus potential to be used as nutricine or prebiotic. The ratio of mannose to galactose could affect the physicochemical properties of the mannose-based polysaccharides and also may affect their physical ability to bind pathogenic bacteria. Therefore, it is urgent information to know the polysaccharides profile of other palm nuts [1].

The role of mannose-based polysaccharides as a substitute for growth-stimulating antibiotics to optimize body weight gain and minimize mortality in poultry has been long believed, especially mannose-based polysaccharides from yeast and coconut [2,4]. The role of polysaccharides from yeast as mycotoxin binding and cholesterol binding has also been reported [5,6]. For this reason, the study of polysaccharides from coconut and salak (Palmae) is expected to replace the function of mannose-based polysaccharides from yeast that is already available in the market. A study was conducted to determine the effect of polysaccharides from coconut and salak in an attempt to replace antibiotics for poultry.

2. Materials and methods

2.1. Extraction of palm nut polysaccharides
Extraction of polysaccharides from coconut and salak nut was based on the method of Kusakabe and Takashi (1988) as the authors developed a method to extract mannose-based polysaccharides from coconut meal [7]. The oils from coconut and salak were extracted to have oil-free coconut meals and salak meals. A total of 16 liters of 20% NaOH concentration were added to 2 kg of either oil-free coconuts and salak meals in a plastic bucket. The mixture between the substrates and NaOH was occasionally stirred for a day at room temperature. To get the filtrate, the slurry was filtered by using a cloth bag. The collected filtrate was neutralized with 12 N sulfate acids until the pH solution was about 5.5. Centrifugation was done to get the resultants. To remove salts, the resultants were dialyzed against tap water. The leftover was a polysaccharide, particularly mannose-based polysaccharides.

2.2. Animals and cages
A total of 140-day old unsexed broiler Cobb chicks were used as experimental animals. The birds were offered sugar liquid immediately upon arrival. The birds were distributed into 5 brooders for seven days after individually weighed. To have a suitable temperature for the chicks during brooding, the brooders with the size of 1.0×1.0×0.5 m³ were electrically heated using a 60-watt bulb. On day three, all the birds were vaccinated against New Castle disease. The broilers were transferred into 20 experimental pens on day eight. The pens were equipped with a plastic feeder and drinker, placed inside each pen. The drinkers and pens were kept clean throughout the study.

2.3. Experimental diets and feeding
The basal diets (table 1) used in this study were formulated by using UFFF software [8]. All the feed ingredients were purchased locally and mixtures of the feed ingredients were done by using a horizontal feed mixer. The use of any feed additives containing antibiotics was avoided. During the first three weeks, the broilers were fed the starter diets and grower diets from weeks 4 to 6. On days 15, 17, 19, and 21, the birds were challenged against Escherichia coli with a concentration of 1.5×10⁸ CFU/ml. These pathogenic
bacteria were kindly provided by health laboratory, Undata hospital, Palu, Central Sulawesi. A total of 5 mL of *E.coli* containing fluid was diluted in 100 mL tap water and offered to birds after 6 hours without drinking. The experimental diets (table 2) and drinking water were provided ad-libitum. The diets were topped up two times a day; at 07.00 in the morning and 16.00 in the afternoon.

### 2.4. Digestibility study

On day 35, two broilers from each replicate pen were randomly selected and placed in the individual metabolism pen for digestibility study. The metabolism pens were equipped with a drinker and feeder located in the front of the pen. A plastic tray with the same size as the metabolic pen was placed underneath each pen to accommodate the fecal discharges. From day 39 to 41, excreta were collected and feed intake was recorded daily. Before weighing the collected excreta, any contamination such as feather and feed particles was discarded by hand-picking. The total excreta was oven-dried for 48 hours at 50°C to determine their dry matter content [9]. Dry matter digestibility was calculated based on the total fecal collection method, according to the procedure of Kong and Adeola [10].

| Table 1. Basal diets. |
|----------------------|
| Feed ingredients     | Starter diet (%) | Grower diet (%) |
|----------------------|------------------|-----------------|
| Full fat             | 24.99            | 18.97           |
| Soybean meal         | 60.20            | 62.10           |
| Maize                | 10.00            | 11.00           |
| Fish meal            | 3.38             | 4.00            |
| Coconut oil          | 1.03             | 0.92            |
| Dicalcium phosphate  | 0.07             | 0.66            |
| Salt                 | 0.15             | 0.55            |
| Methionine Lysine    | 0.05             | 0.11            |
| Premix               | 0.20             | 0.20            |
| Calculated nutrients: |                   |                 |
| Crude protein        | 23.13            | 21.00           |
| Crude fiber          | 3.50             | 3.6             |
| Metabolizable energy | 13.39            | 13.39           |
| (MJ/kg) Lysine       | 1.10             | 1.00            |
| Methionine + cysteine| 0.90             | 0.79            |

| Table 2. Experimental diets. |
|-----------------------------|
| Treatments                  | Replications | Birds |
| Control basal diet (T1)     | 4            | 7     |
| Basal + Antibiotic avilamycin (T2) | 4    | 7     |
| Basal + Actinogen; commercial yeast oligosaccharides (T3) | 4 | 7 |
| Basal + Salak polyolsaccharydes (T4) | 4 | 7 |
| Basal + Coconut polysaccharydes (T5) | 4 | 7 |

### 2.5. Carcass and bursa of Fabricius measurement

At the end of the trial, the birds were fasting for 6 hours to empty the tract digestive fill. The broilers were then weighed and slaughtered. The slaughtered birds were dressed by removing the feathers, skin, neck, shank, and digestive organs [11]. The dressed broilers were weighed for carcass percentage and bursa of Fabricius from each killed broilers were individually weighed.
2.6. Statistical data and analysis
The study used a completely randomized design with 5 treatments and 4 replicate cages. Data of the monosaccharides profile of the polysaccharides were descriptively analyzed. Data of body weight gain, feed consumption, FCR, excreta dry matter, dry matter digestibility, carcass, and bursa of Fabricius were analyzed using analysis of variance [12]. Any differences found by the statistical analysis were further tested by the least significant difference test using a Minitab statistical program [13].

3. Results and discussion

3.1. Results
Data on the profile of sugar content in palm plants can be seen in table 3. Data on the effect of the treatments on growth performance, dry matter excreta, dry matter digestibility, carcass percentage, and bursa of Fabricius were shown in table 4 and table 5. Treatments affected significantly body weight gain (P<0.001), feed intake (P=0.022), FCR (P<0.001), bursa of Fabricius (P<0.001), Carcass percentage (P<0.001), and excreta dry matter (P<0.001). Dry matter digestibility, on the other hand, was not affected by the treatment diets.

Table 3. The profile of sugars on polysaccharides in coconut and salak fruit.

| Polysaccharides    | Mannose | Glucose (ppm) | Arabinose (ppm) |
|--------------------|---------|---------------|-----------------|
| Coconut meal       | 6233    | <10.5         | <27.4           |
| Actigen            | 1000    | 23067         | 685             |
| Salak fruit        | <31     | 12632         | <27.4           |

Table 4. Effect of treatment on weight gain, feed intake, and FCR

| Treatments                  | Weight gain (g) | Feed intake (g) | FCR    |
|-----------------------------|-----------------|-----------------|--------|
| Basal                       | 899^b           | 1739^b          | 1.93^a |
| Basal + avilamycin          | 1062^a          | 1851^a          | 1.74^b |
| Basal + Actigen             | 1049^a          | 1816^ab         | 1.73^b |
| Basal + Coconut polysaccharide | 1065^a      | 1827^ab         | 1.73^b |
| Basal + Salak polysaccharide | 1048^a          | 1809^ab         | 1.73^b |
| P Value                     | >0.001          | 0.022           | >0.001 |
| SEM                         | 11.38           | 11.7            | 0.016  |

Commercial product of yeast oligosaccharides; FCR: feed conversion ratio; SEM: Standard error means

3.2. Discussion
Actigen is the product of Alltech Biotechnology, acting as a natural alternative to antibiotics. This product contains a mannan-rich fraction [14]. The analysis of monosaccharides fraction indicates a high quantity of mannose found in Actigen, along with a high concentration of glucose. It might be that mannan in this product is in the form of glucomannan. The efficacy of this product in improving the growth performance of broiler chickens has been well discussed by Spring et al [14].

The high content of mannose in coconut meal indicates that the majority of polysaccharides in coconut meal are in the form of mannan. According to Balasubramaniam (1976), the main component of coconut
polysaccharides was 26% mannan, 61% galactomannan, and 13% cellulose. *E. coli* has fimbriae that bind mannose [15]. Accordingly, the high concentration of mannose in coconut meal is the potential to be used as a source of prebiotic [2].

**Table 5.** Effect of treatments on excreta dry matter, dry matter digestibility, carcass, and bursa of Fabricius percentages.

| Treatments                        | Parameters (%)                  |
|-----------------------------------|---------------------------------|
|                                   | bursa of Fabricius | Carcass | DM Excreta | DM Digestibility |
| Basal                             | 0.218 ± 0.001       | 67.4 ± 0.2 | 14.9 ± 0.2 | 71.8 ± 0.2 |
| Basal + avilamycin                | 0.187 ± 0.001       | 70.7 ± 0.2 | 20.3 ± 0.2 | 80.1 ± 0.2 |
| Basal + actigen                   | 0.193 ± 0.001       | 70.8 ± 0.2 | 20.7 ± 0.2 | 81.2 ± 0.2 |
| Basal + coconut polysaccharide    | 0.185 ± 0.001       | 70.6 ± 0.2 | 20.7± 0.2  | 80.5 ± 0.2 |
| Basal + salak Polysaccharide      | 0.202 ± 0.001       | 70.6 ± 0.2 | 21.3 ± 0.2 | 80.3 ± 0.2 |
| P Value                           | >0.001              | >0.001   | >0.001     | 0.178         |
| SEM                               | 0.392               | 0.414    | 0.159      | 0.967         |

DM: Dry matter, SEM: Standard error mean

Interestingly, glucose is the main carbohydrate present in Salak fruit. There are two possibilities of the polysaccharides in salak fruit, cellulose or glucan. This speculation is based on the fact that the polysaccharides of salak are in the form of a polymer of glucose. A study on salak carbohydrate has been done by Lestari et al (2013) who identified that the main component of polysaccharides was pectin [16]. The different monosaccharides profiles of salak fruit might be due to the different methods used, particularly the solvent. Due to the difference monosaccharides profile of salak to yeast and coconut, those fruits might behave differently in the physicochemical properties.

Feeding the broilers challenged with *E. coli* with polysaccharides from coconut and salak fruit increased body weight gain of broiler chickens to the same body weight gain as in broiler chickens fed the diet supplemented with antibiotic avilamycin. These findings might indicate that polysaccharides from coconuts and salak fruit can replace growth-stimulating antibiotics. The birds fed the control basal diet had the lowest body weight gain when the birds were challenged against *E. coli*. The mechanism of the increased body weight gain due to the addition of polysaccharides from coconut and salak fruit might be the same as the action of the yeast polysaccharide (*Saccharomyces cerevisiae*) [16,17]. Polysaccharides from yeast (mannan yeast) can bind pathogenic bacteria and flush them out of the digestive tract. This condition could maintain the digestive tract health when yeast mannan was present in the broiler diets [4]. However, polysaccharides from coconut, salak, and actigen (commercial mannan from yeast) could enhance the body weight gain of broiler chickens kept for 4 weeks. These findings were promising and potential regarding the use of polysaccharides from Palmae as a growth-stimulating additive.

The effect of treatments on feed consumption was significant in which birds fed the diet supplemented with Avilamycin consumed more than those birds fed the control basal diet. This finding was in contradiction to the previous finding of Chowdhury et al (2009), who stated that the action of avilamycin in the diet did not increase feed intake [18]. The feed conversion ratio of birds fed the diet supplemented with feed additives was better than the control diet. Since the effects of the experimental diet on body weight gain and FCR were significant, it can be said here that the addition of polysaccharides from coconut meal and salak fruit in the diet was possibly effective in improving the quality of feed in this current study when the broilers were challenged against *E. coli*.

The high percentage of the carcass in broiler chickens fed diets containing antibiotics, polysaccharides from yeast, coconut and Salak might be related to the high body weight gain as the carcass percentage
depends a lot on carcass weight. Bursa of Fabricius of *E. coli*-challenged birds fed the control basal diet was larger than those birds fed the other diets [19]. This possibly indicated that the pathogenic bacteria of *E. coli* infect the immune organ of the bursa of Fabricius. The antibiotic Avilamycin and prebiotic Actigen are commercial products functioning to protect the animals from pathogenic bacteria. The coconut polysaccharides and salak polysaccharides might behave the same as yeast polysaccharides (Actigen) at least according to the physical response of the bursa of Fabricius as an immune organ.

4. Conclusion
The addition of polysaccharides from coconut and salak increased body weight gain and FCR to the same level of body weight gain of birds fed the Avilamycin antibiotic or Actigen, the commercial yeast polysaccharides. The feed intake of broilers fed the Avilamycin containing diet was higher than the feed intake of control birds. The birds fed the control basal diet produced lower excreta dry matter and carcass percentages. The bursa of Fabricius of control birds was larger than the birds fed the Avilamycin, Actigen, and coconut polysaccharide-containing diets. Dry matter digestibility was not affected by treatment diets.

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