Effects of addition chitosan-oligosaccharide of snail shell in the diet on quail (*Coturnix coturnix japonica*) performance and carcass characteristics

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Abstract: It is well documented that feed additives improve livestock productivity through improving feed intake, feed efficiency, and livestock health status. Oligosaccharide is categorized as a prebiotic additive that can modify the microbial ecosystem in the small intestine by creating an environment that is more favorable for nonpathogenic bacteria’s growth. Current research investigates the effects of chitosan-oligosaccharide (COS) from snail shells in the diet on quail performance and carcass characteristics. A total of 100 female DOQ were placed in a Completely Randomized Design, five treatments and four replicates with five birds for each replicate and were kept under standard management conditions for six weeks. Basal diet consisted of corn, rice bran, soybean, fish meal, coconut oil, minerals, methionine, and lysine, and was mixed in such a way to achieve 22% protein. Treatments were the basal diet without feed additive (T1) as the negative control, the basal diet with 28 ppm preparation of lactate acid bacteria, LAB (T2) as the positive control, the basal diet with 200 ppm of COS (T3), the basal diet with 400 ppm of COS (T4), and the basal diet with 600 ppm of COS (T5). The birds were having free access to the diets and drinking water. Bird's weight and feed intake were recorded weekly. At the end of the experiment, two birds from each cage were selected and slaughtered for carcass assessment and weight digestive organ. The variables are feed intake, live weight gain, feed conversion ratio, carcass yield, breast meat weight, and organ weight. The data were analyzed by using Anova and comparison tests using the Duncan test. The results revealed that the addition of COS in the diet significantly reduced (P<0.05) feed intake, carcass yield, and dressing percentage and improved (P<0.05) feed conversion ratio, liver, and two ceca (P<0.05). Other parameters were not different (P>0.05) between treatments. Therefore, it could be concluded that the inclusion of chitosan-oligosaccharide (COS) in the diet improved quail performance by enhancing feed efficiency.

1. Introduction
The inclusion of feed additives in the diet is intended to stimulate feed intake, improve the health status of livestock, elevate production, and in turn, increase feed efficiency. However, the use of synthetic additives such as antibiotics is no longer recommended in the livestock production process because the use of antibiotics has an impact on livestock performance and its product. Antibiotics
create resistance of the digestive tract micro biota of livestock and environmental pollution [1]. Therefore, it urgently requires alternative additives to enhance animal production without any side effects in livestock and the environment. Currently, studies have been the focus on the additive that can promote gut function to a favorable environment for better growth of the nonpathogenic bacteria and suppressing the growth of pathogenic bacteria through providing prebiotics additive in the diet.

Prebiotics are nondigestible feed ingredients that benefit the host by altering the composition and metabolism of digestive tract microbiota selectively [2]. One type of prebiotics that are used in animal diets is chitosan-oligosaccharide or chito-oligosaccharides (COS). Guan et al (2019) reported that the use of chitosan and its derivatives (COS) has a beneficial effect from biological aspects, for example, as an antibacterial, antioxidant, suppresses cholesterol, and as an immunomodulator [3]. Studies in different animals revealed that COS improved growth performance, improved live weight, feed intake, and feed conversion ratio [4–7]. On the contrary, the inclusion of COS in diet did not affect broiler performance, carcass characteristics, and breast meat [7–9].

The COS is generated from the natural substance of chitin, found in hard-shelled animals such as crab, shrimps, and snail [10]. Furthermore, Ridhay et al (2019) and Nurhaeni et al (2019) [10,11] have been working on locally available snail field (Pila ampullacea) to produce chitosan and it enzymatically hydrolyzed to yield hydrolyzed-chitosan (COS). This end product has been proven to have antibacterial activities in vitro study. Therefore, the current study was conducted to investigate the efficacy of inclusion COS generated from the snail field in the diet on quail performance and carcass characteristics.

2. Materials and method

The raw materials of chitosan oligosaccharide (COS) were taking from snail shells (Pila ampullacea) and the process to get chitosan-oligosaccharide was done according to the procedure as described in Ridhay et al (2019) and Nurhaeni et al (2019) Briefly, dried snail shells were grounded using pastel mortar, then sieved with a 100 mesh sieve [10,11]. Powder shells were demineralized and deproteinized to get chitin. Chitin was mixed with a particular amount of NaOH. The mixtures were agitated for 3 h at the 120'C and filtered. The sediment formed after filtration processes was chitosan. Chitosan was then enzymatically hydrolyzed using α-amylase enzyme with adjusted pH and temperature. The characteristics of hydrolyzed chitosan (COS) were explained in Ridhay et al (2019) and Nurhaeni et al (2019) and this product of COS was used for feed additive in the present study [10,11].

Basal diet consisted of corn, rice bran, soybean, fish meal, coconut oil, premix, salt, methionine, and lysine (table 1). The basal diet was mixed in such a way as to achieve 22% protein as recommended by the NRC [12]. Experimental diets were the basal diet mixed with COS at various proportions or mixed with 28 ppm of preparation lactate acid bacteria, LAB as the positive control. They are basal diet without feed additive (T1) as the negative control, the basal diet with 28 ppm of preparation lactate acid bacteria, LAB (T2) as the positive control, the basal diet with 200 ppm of COS (T3), the basal diet with 400 ppm of COS (T4) and the basal diet with 600 ppm of COS (T5).

The birds were kept in wire cages for six weeks. Each cage was equipped with a feeding and drinking trunk, and lamps for heating and lighting. A total of 100 of one-day-old female quail (DOQ) were allocated to a completely randomized design (CRD) within five treatments and four replicates per treatment with five birds per cage, and therefore there were 20 unit cages. Experimental diets and drinking water was given ad libitum throughout the experimental period. The variables included feed intake (FI), live weight gain (LWG); feed conversion ratio (FCR), carcass yield, and organ weight.

The protocol applied in this study has been approved by the animal ethics committee of the Faculty of Animal Husbandry and Fishery, Tadulako University Palu, Indonesia. Feed intake and bird's weight were recorded weekly. Two birds from each cage were selected and slaughtered for carcass yield and organ weight. Data from variables were analyzed using analysis variance and Duncan test for comparison mean test [13].
### Table 1. Basal diet composition

| Feed Ingredients | %   |
|------------------|-----|
| Corn             | 59.00 |
| Soybean meal     | 20.00 |
| Fish meal        | 16.00 |
| Rice bran        | 4.00  |
| Palm oil         | 0.15  |
| Salt             | 0.02  |
| Lime stone       | 0.13  |
| Premix\(^1\)     | 0.30  |
| Lysine           | 0.20  |
| DL-Methionine    | 0.20  |
| Total            | 100   |

#### Calculated composition (%)

- Metabolizable Energy (kcal kg\(^{-1}\)) : 3,260
- Crude Protein : 22.44
- Crude Fiber : 2.86
- Calcium : 1.01
- Phosphorus : 0.28
- Lysine : 1.10
- Methionine : 0.50

\(^1\) Supplied the following per kilogram: Calcium 32.5%; Phosphor 10%; Iron 6 g; Mangan 4g; Iodine 0.075g; Copper 0.3g; Zinc 3.75g; vitamin B12 0.5 mg; vitamin D3 50,000 IU; vitamin A 1,200,000 IU; vitamin B1 200 mg; vitamin B2 500 mg; Vitamin B6 50 mg; vitamin C 2,500 mg; Ca-D-pantotenate 600 mg; Niacin 4000 mg; Methionine, 3,000 mg; Lysine, 3,000 mg; Santoquin, 1,000 mg; Zinc bacitracin, 2,100 mg.

### 3. Results and discussion

The quail in the present study did not perform as expected. In general, their performances (table 2) are lower compared with Cakir et al [14], Sahin et al [15] and Tufan et al [9]. There was a significant (P<0.05) effect of inclusion of feed additive in the diet on feed intake and feed conversion ratio. Quail in the lactic acid bacteria (LAB) group significantly consumed less feed compared to the negative control (P<0.05), meanwhile quail in the COS group also consumed less feed compared to the negative control group (P>0.05) and no significant differences were detected in LWG between treatments. Additionally, the results showed that the measured parameters of quail consuming COS had the same response pattern to the LAB treatment group. Previous studies found that the inclusion of different levels of COS in the diet produced no effect on LWG of broiler chicken or quail [8,9,16], but Li et al (2007) and Zhou et al (2009) revealed a positive effect of COS on LWG of broiler chicken [6,7]. The reduction in feed intake, however, improved FCR in the additive group compared with the negative control group. Improvement of FCR can be associated with enhancement of small intestine surface area through increased height and width of villi in the ileum and jejunum (data are not shown), leading to the increase in digestibility and absorption of nutrients in the digestive tract. This finding is supported by Zhou et al [17] found enhancement of dry matter and nitrogen digestibility and growth performance of weaned pig consumed diet containing COS at the rate of 2g/kg. Other studies by Tufan et al (2015) reported that the inclusion of COS in the quail diet produces no effect on FCR [9]. Inconsistent results in growth performance parameters between this study and other studies could be associated with differences in molecular weight of COS, deacetylation degrees of COS, dose rate, species, feeding period, and basal diet composition.

The mechanism of decreasing feed intake in the current study can be related to the pressure on the digestive tract, as chitosan generally form high viscous solution in the digestive tract of animal [18] that
create distention in the animal's duodenum region [19]. Therefore this is allowing satiety state of the animal. Chitosan has also been reported to elevate serum leptin in pigs [20], and leptin has a role as an appetite suppressant in the central nervous system [21]. An additional mechanism is the regulation of feed intake in response to the blood glucose levels, feed intake will increase when blood glucose levels decrease, and vice versa and the administration of chitosan and its derivate (COS) increase blood glucose levels in pig [22].

Table 2. Initial weight, liveweight gain, feed intake, feed conversion of quail

| Variables | Treatments | T1 | T2 | T3 | T4 | T5 | SEM |
|-----------|------------|----|----|----|----|----|-----|
| Initial weight (g) | 6.90 | 6.49 | 7.20 | 7.00 | 6.85 | 0.13 |
| LWG (g) | 95.66 | 90.42 | 96.14 | 89.74 | 95.94 | 1.77 |
| FI (g) | 377.30<sup>a</sup> | 348.30<sup>b</sup> | 380.35<sup>a</sup> | 358.15<sup>ab</sup> | 368.40<sup>ab</sup> | 4.31 |
| FCR | 3.82<sup>a</sup> | 3.56<sup>b</sup> | 3.50<sup>b</sup> | 3.65<sup>ab</sup> | 3.55<sup>b</sup> | 0.04 |

LWG: liveweight gain, FI: feed intake and FCR: feed conversion ratio. SEM: standard error mean. On the same line different letters indicate significant differences (P <0.05).

As presented in table 3, addition feed additives in the diet significantly reduced (P<0.05) carcass yield, dressing percentage, and carcass cut were not different between treatments (P>0.05). This finding agrees with Tufan et al. (2015) who reported that addition COS in the quail diet resulted in a non-significant reduction in carcass yield and dressing percentage [9]. Meanwhile, Sahin et al. (2008) revealed that inclusion prebiotic, probiotic, or combination prebiotic-probiotic in diet produced a non-significant increase in carcass yield and dressing percentage [15]. The decreased in carcass parameters in the present study can be associated with significantly enhancing liver weight and two secca weights, as presented in table 3. The carcass yield is also partly determined by the low live weight gain of the quail, as shown in table 2.

The weight of the liver and two ceca significantly increased (P<0.05) by the inclusion of COS in diet, while other organs were not affected (P>0.05) by feed additive (table 3). A similar finding has been reported by Zhou et al. (2009) that inclusion of COS in broiler chicken diet enhanced the liver weight [7]. Simultaneously, Cakir et al. (2008) found a non-significant enhancement of liver weight in quail given prebiotic combination with probiotic [14]. Moreover, the liver is a very dynamic organ in poultry, which functions for synthesis, metabolism, excretion, and detoxification process in the body [23]. The liver plays an important role in digestion and metabolism, regulating production, storing, and releasing carbohydrates, lipids, and proteins. Since there is a process of nutrient synthesis or storage in the liver, then transported to plasma for storage, it improves the liver size, indicating a function of high metabolic activity has occurred [23]. Another mechanism that can describe the increase in liver weight at low feed intake in the present study is that low feed intake is usually followed by an increase in water intake [24,25] and digest becomes watery, resulting in low digesta viscosity. This condition results in increased absorption of fat and fat-soluble vitamins, then fat accumulation in the liver. Hence the increased activity of hepatocyte enzyme causing hepatic hyperplasia and hypertrophy, allowing increased liver weight.

Ceca's weight was significantly elevated by the inclusion of COS in the present study. This finding agrees with Oyarzbal and Conner (1996) that increased ceca in prebiotic-treated broiler compared to the control group [26]. The significant increase in two ceca can be attributed to the rise in the residence time of the material due to increased fermentation activity resulting from prebiotics/probiotics in the diet. Increased anaerobic fermentation activity in ceca directly increases short-chain-fatty acid (SCFA) production and is generally dominated by acetic acid components [27]. However, the concentration and composition of SCFA are determined by the type of material available in ceca [28]. An increase in fermentation activity results in an improvement of SCFA products.
Furthermore, SCFA has a positive effect on host livestock, i.e., providing additional energy available and creating an acidic intestinal environment that suppresses the growth of pathogenic bacteria so that more nutrients are available for livestock growth. This is also one mechanism that explains in the improvement of the FCR as described earlier. These facts all justify the advantages of inclusion COS in the diet, and this is in line with the recommendations of Yadav and Jha (2019) suggesting the addition of feed additives singly or a combination of feed additives in the diet to achieve an optimal environment and microbiota population in the digestive tract for better growth in livestock [2].

4. Conclusion

The addition of chitosan-oligosaccharide (COS) in the diet significantly reduced intake, carcass yield and dressing percentage, improved feed conversion ratio, liver weight, and two ceca. Other parameters did not influence significantly by the addition of COS feed additive. Therefore, it could be concluded that the inclusion of chitosan-oligosaccharide (COS) in the diet improved quail performance by enhancing feed efficiency.

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**Table 3. Carcass characteristics and digestive organ of quail**

| Variables       | T1    | T2    | T3    | T4    | T5    | SEM  |
|-----------------|-------|-------|-------|-------|-------|------|
| Carcass yield (g) | 81.57<sup>a</sup> | 70.00<sup>b</sup> | 80.88<sup>a</sup> | 72.38<sup>bc</sup> | 77.38<sup>ac</sup> | 1.37 |
| Dressing percentage (%) | 77.15<sup>a</sup> | 73.77<sup>b</sup> | 76.03<sup>ab</sup> | 74.42<sup>ab</sup> | 75.93<sup>ab</sup> | 0.37 |
| Breast meat (% of carcass) | 40.28 | 38.97 | 39.52 | 39.03 | 39.60 | 0.42 |
| Legs (% of carcass)   | 23.19 | 23.69 | 23.22 | 22.65 | 24.11 | 0.34 |
| Wings (% of carcass)  | 9.05  | 9.51  | 8.83  | 8.86  | 8.62  | 0.14 |
| Gizzard (g)           | 3.45  | 2.85  | 3.39  | 3.93  | 3.29  | 0.15 |
| Liver (g)             | 1.81<sup>a</sup> | 1.79<sup>a</sup> | 2.14<sup>b</sup> | 1.93<sup>ab</sup> | 2.10<sup>b</sup> | 0.04 |
| Gut (g)               | 4.04  | 3.98  | 4.21  | 4.73  | 4.28  | 0.13 |
| Two ceca (g)          | 0.66<sup>a</sup> | 0.76<sup>a</sup> | 0.94<sup>b</sup> | 0.93<sup>b</sup> | 0.92<sup>b</sup> | 0.03 |

SEM, standard error mean. On the same line different letters indicate significant differences (P < 0.05).
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