The effect of grit administration on the macroscopic and microscopic appearance of starter phase broilers digestive tract

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Abstract. Digestive tract has a key role in poultry growth due to its function in absorbing nutrients. Grit is one of feed ingredients widely used to accelerate grinding process. The purpose of this study was to determine the effect of grit administration on broiler chickens in the starter phase from age 1 to 21 days on the macroscopic and microscopic appearance of the digestive tract. The study design consisted of four combinations and five replications group from 200 broilers, with 10 broilers in each group. The treatments given were 0% grit; 0.5% grit of BW (body weight); 1% grit of BW; 1.5% grit of BW. The results showed that grit administration affected the macroscopic appearance of broilers digestive tract. Grit administration had a significant effect (P<0.05) on gizzard weight, duodenal length, jejunum length, ileum length, total intestinal length and total intestinal surface of broilers and had no significant effect (P>0.05) on the width of the small intestine. The results showed that grit administration affected the microscopic appearance of broiler chicken intestines. Grit administration had a significant effect (P<0.05) on the basal width and surface area of the small intestine villi and had no significant effect (P>0.05) on the villi apical width, villi height, and cryptic depth. In conclusion, grit administration can improve the macroscopic appearance of digestive tract and can improve the surface area of the small intestine villi with the optimal concentration 0.5% of body weight.

1. Introduction
Broiler chickens are meat-producing poultry that have a rapid growth rate in a short time, so they have great potential in commercial business [1]. Digestive tract is one of the organs that provides nutrition to the body. Every food that enters the body will be converted into energy which will be used by the body to survive, grow and produce. The faster the digestive tract can function properly in a chick, the faster the chick can digest and use the nutrients contained in feed to grow and develop according to their genetic potential.

Grit is small stones like gravel, which are consumed by chickens which can be found in gizzard. Grit as a tooth substitute is used by poultry to help gizzard in breaking large particles from the feed into smaller particles.

According to Ling et al. [2] grit administration can improve the performance of growth and development of the digestive tract. The increased growth of the digestive tract includes an increase in relative weight or length of the proventriculus, gizzard and duodenum.
Grit administration to broilers did not show any general adverse effect on performance parameters of the digestive organs. Grit administration is able to affect the volume of the gizzard lumen which is enlarged as a result of muscle activity which also increases due to stimulation of grit [3]. Likewise Sabuna et al and Ling et al [2,4] and showed that grit can improve gizzard performance and digestive organ growth, including gizzard and intestines growth.

Grit is widely used in livestock industry is either in digested form or not. Previous research used a mixture of digested and undigested grit, including limestone, shells and gravel with a ratio of 30:30:40. In this study, grit composed of limestone and shellfish with a ratio of 50:50. The purpose of this study was to determine the effect of grit stone administration to starter phase broilers on the macroscopic and microscopic appearance of the digestive tracts.

2. Materials and method
The research was conducted from March to May 2020, at the Animal Farms of Faculty of Animal Husbandry, University of Muhammadiyah Malang as a place to raise broiler chickens, Bioscience Laboratory as a place for preparing small intestine preparations, and the Laboratory of Animal Husbandry & Nutrition, University of Muhammadiyah Malang as a place for observation of the digestive tract.

Two hundreds broilers were used from the Cobb strain. They were divided into 4 treatment groups with 5 replications. This research was conducted experimentally using a complete randomized design method. Macroscopic variables measured from the digestive organs of broilers included gizzard weight, duodenal length, jejunum length, ileum length, small intestine width and intestinal surface area (cm²). Microscopic variables included villi height, apical villi width, basal villi width, crypt depth and the surface area of the small intestine villi of broiler chickens.

Variable measurements were carried out when the chickens were 15 days old. Measurement of the surface area of the villi was carried out in the small intestine of the ileum. Observation of the structure of the small intestine included crypt depth, villi height, basal width and apical width using an Olympus Binocular microscope with an MDCE-5C camera. Taking pictures using Optilab and measuring using the Image Raster application, with a magnification of 100x. The small intestinal tissue was cut with a thickness of 4 μm. Measurement of the structure of the small intestine in one cross section was carried out on 3 randomly selected villi. The calculation of the surface area of the villi was carried out using the Iji [5] method. Data analysis used unidirectional pattern variance analysis (One way ANOVA), if there is a significant difference, it is followed by the Least Significant Difference test.

3. Results and discussion
Grit administration seems to stimulate growth and improve the performance of the digestive organs as listed in table 1.

| Table 1. Result of macroscopic appearance of broiler digestive organs. |
|---------------------------------------------------------------|
| **Macroscopic appearance** | **Treatment groups** | **P0** | **P1** | **P2** | **P3** |
| Gizzard weight (g) | 19.708 ±6.278<sup>a</sup> | 25.786 ±1.800<sup>b</sup> | 29.392 ±2.875<sup>c</sup> | 22.312 ±5.058<sup>a</sup> |
| Duodenum length (cm) | 22.840 ±6.082<sup>a</sup> | 29.000 ±2.706<sup>b</sup> | 26.200 ±2.033<sup>c</sup> | 23.360 ±0.563<sup>a</sup> |
| Jejunum length (cm) | 70.460 ±9.682<sup>b</sup> | 72.620 ±5.209<sup>b</sup> | 69.800 ±6.330<sup>b</sup> | 58.720 ±3.992<sup>a</sup> |
| Ileum length (cm) | 58.800 ±9.670<sup>b</sup> | 68.560 ±7.539<sup>a</sup> | 65.000 ±2.162<sup>b</sup> | 55.000 ±4.039<sup>a</sup> |
| Total intestinal length (cm) | 152.100 ±22.020<sup>a</sup> | 171.180 ±14.559<sup>a</sup> | 161.000 ±6.890<sup>a</sup> | 137.080 ±2.393<sup>a</sup> |
| Intestinal width (cm) | 1.380 ±0.258 | 1.560 ±0.270 | 1.30 ±0.244 | 1.460 ±0.114 |
| Intestinal surface area (cm²) | 208.492 ±41.336 | 266.716 ±47.388 | 209.340 ±199.986 | 199.986 ±44.135 |

Different superscripts on the same row show significant differences (P<0.05).

In table 1, it can be seen that grit administration can increase gizzard weight and increase the length of the digestive tract. These results support the research conducted by [4], that addition of grit can increase the weight of the gizzard and lengthen the digestive tract. Meanwhile [6], showed that feed supplemented with insoluble grit improved gizzard development and broiler health.
The administration of grit to starter phase broilers had a significant effect (P<0.05) on the length of the small intestine of chickens. It can be seen in the graph above that grit at group P1 (grit 0.5% of body weight) and P2 (grit 1% of body weight) lengthened the intestines more than P0 (0% grit). However, the higher concentration of grit in broiler chicken decrease intestinal growth. The average length of the small intestine at group P3 (grit 1.5% of body weight) appeared to be shorter than P0. The possibility is that the concentration of 1.5% grit is too high for broilers at starter age. In addition, exaggerated administration of grit could disturbed or even damaged the growth of the digestive organs.

The optimal concentration giving the best effect in this study was seen in group P1, with grit 0.5% of body weight. The average length of the duodenum, jejunum, and ileum of P1 had the longest size compared to the other 3 treatment groups. P2 (grit 1% of body weight) has average length above P1 (control), meanwhile P3 (grit 1.5% of body weight) has average length of intestine that is far below P1 (control). Grit administration to starter phase broilers had no significant effect on intestinal width (P>0.05).

Table 2. Result of microscopic appearance of intestinal villi.

| Microscopic appearance   | Treatment groups |
|--------------------------|------------------|
|                          | P0               | P1               | P2               | P3               |
| Villi height (μm)        | 370.493 ±103.846 | 459.307 ±85.581  | 434.913 ±139.816 | 315.420 ±77.651  |
| Apical width (μm)        | 24.54 ±5.50      | 26.09 ±9.13      | 22.45 ±3.39      | 30.19 ±13.25     |
| Basal width (μm)         | 56.43 ±15.621    | 84.773 ±15.251   | 54.688 ±11.840   | 57.713 ±16.731   |
| Crypt depth (μm)         | 86.05 ±16.01     | 102.85 ±18.16    | 95.72 ±30.91     | 119.45 ±28.35    |
| Villi surface area (μm²) | 1334.06 ±515.885 | 2347.397 ±132.312 | 1634.634 ±598.262 | 1319.622 ±242.804 |

Different superscripts on the same row show significant differences (P<0.05).

Table 2 showed that grit administration had a significant effect on the basal width of the intestine and the surface area of the villi (P<0.05). The highest surface area of villi was observed in treatment P1 (0.5%) with 2,347,397 μm², higher than group P0 (0%), P2 (1%), and P3 (1.5%).

In this study, administration of grit made from limestone and shellfish with a ratio of 50:50 was proven to increase the surface area of the small intestine villi. It was suspected that the grit consumed by chickens from day one (DOC) was able to stimulate the growth small intestine.

The use of limestone and shellfish for grit made it possible for poultry to digest. The main function of grit in the digestion of broiler is to mechanically grind food particles. The limestone and shellfish will be dissolved in the digestion process of broilers. In the digestion organ, grit will be moved from the gizzard to the small intestine and will stimulate the development of the small intestinal villi.

Grit that is not absorbed will passes through the intestine (large and small intestine) and cause friction against the intestinal villi so that it stimulates the intestinal villi to expand and makes them increase in size, namely the weight and length of the intestine. Bennett et al, (2002) stated that the size of the intestine increases when given grit compared to not given grit [7]. The length of the various parts of the digestive tract of poultry is not a static quantity. Changes can occur because influenced by the type of feed consumed followed by changes in the number of intestinal villi. According to Sugito et al., (2007), micro villi are present on the surface of the villi as cytoplasmic protrusions which can increase absorption efficiency [8]. The larger the surface area of the intestinal villi, the greater the chance of absorption from the digestive tract. According to [4], the use of grit up to a level of 0.75% of the body weight of native chickens during the growth phase increases the development of the digestive tract and carcass bone weight, while according to [4,9], daily feed intake, daily body weight gain and feed conversion ratio of local chicken fed grit were significantly higher and better (P<0.05) than those without grit administration.
Figure 1. Cross section of the small intestine villi (from left to right; P0, P1, P2 and P3).

Figure 1 showed a cross section of the small intestine villi in broilers from each treatment groups. The appearance of the intestinal villi in treatment P0, P1 and P2 was more prominent than in P3. In P3, the small intestine villi is more difficult to observe since the whole appearance of the villi is not visible that requires high accuracy to observe. It was possible that group P3 was given high concentration of grit that cause damage toward intestinal villi. Excessive consumption of grit caused the digestible grit become undigested due to excessive capacity or consumption.

4. Conclusion
Administration of digested grit made from limestone and shellfish with a ratio of 50:50 to starter phase broilers has a significant effect on the macroscopic performance of the digestive tract which includes gizzard weight, duodenal length, jejunum length, ileum length, total small intestine length and intestines surface area. Grit administration also significantly affects the microscopic image of the small intestine which includes the width of the basal villi and the surface area of the small intestine villi. However, grit administration did not significantly affect intestinal width, apical width, villi height, and depth of small intestine villi crypts. The optimal concentration was given in P1, given grit 0.5% of body weight.

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