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The effects of marble grit supplementation on the performance of broiler chicken

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Abstract

A trial was carried out to investigate the effects of both insoluble and soluble grits supplemented to broiler chicken on the growth performance and gizzard stimulation. Particle size distribution of excreta, grit passage, grit disappearance and AME were also examined. 252 day-old male broiler chickens were raised in 4 pens for 5 days and 192 (> 130g) of them were randomly and equally allocated into 48 quail cages (4 birds in each cage) and maintained on a commercial pelleted diet. 48 cages were divided into 4 groups, which were control group, granite group, zeolite group and marble group. Grit stones were given to their respectively treatment group on day 5 (2 g/bird), 7 (3.75 g/bird), 9 (3.75 g/bird) and 18 (1 g/bird), 19 (1 g/bird) and 20 (1 g/bird) on top of the feed. Remaining grit stone residues were removed and recorded on day 13 and day 21. Bird weight and feed consumption were registered at 5, 11, 13, 18 and 21 days of age. Quantitative sampling of excreta was collected from 5-11, 11-13, 13-18 and 18-21 days of age. These samples were frozen immediately for further analysis. One randomly selected bird from each cage was killed and dissected on day 13, 18, 21 and 22. Full and empty gizzard weight was recorded on all dissection days. The crop was collected on day 21 and 22. Both gizzard content and intestines were frozen immediately for further analysis. The findings showed that marble supplementation impaired the feed intake and weight gain of birds, whereas insoluble grits had no effects on the growth performance of birds. The pH and size of gizzard content were not affected by marble supplementation nor insoluble grits supplementation, whereas birds from granite group had significantly ($p < 0.05$) larger values of gizzard content weight, relative gizzard content and empty gizzard weight than birds from marble group on day 13. The particle size distribution of excreta and AME were not affected by insoluble grits nor marble grit.

Key words: Marble grit, Insoluble grit, Growth performance, Gizzard stimulation, Particle size distribution, AME.

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1. Introduction

Feed cost is reported to account for up to 70% of the total production cost in broiler industry (Abdollahi et al., 2013). The increase of feed ingredient and feed processing cost have brought the challenge to the broiler industry. For this reason, it is of great importance to improve the feed conversion ratio of the broiler chicken. Many efforts have been made in feed formulation and feed form to improve the feed efficiency. Dari et al (2005) detected formulation based on digestive amino acid improved revenue of broiler production. Amerah et al (2007) described that pelleted diets were better in broiler performance compared with mash diets. The pelleting process is widely applied in commercial broiler feed due to its advantages in increasing the feed intake and thereby improve the growth performance and feed efficiency (Abdollahi et al., 2013). Apart from other monogastric animals, broiler chicken has no teeth but a muscular stomach which is called gizzard in the digestive system. The gizzard takes over the function of grinding large and durable feed into smaller particles so that they can be ready for efficient reaction with enzymes working in the digestion (Scott et al., 1956). Xu et al (2015) pointed out that gizzard function was enhanced when birds were fed with coarse feed owing to the intense grinding process. It is generally accepted that poultry benefit from grit eaten which plays an important role as digestive aid (Fuller 1958).

The outcome from previous studies showed that the importance of grit stones in poultry digestion is controversial. It was assumed birds need grit when fed with coarse feed, and it's not necessary to give grit when fed with mash diet. Fritz (1936) discovered the digestibility increased when birds were fed granite. Bennett (2002) reported that feed grit to turkey did not seem to be necessary when turkeys were fed whole grain. Garipoglu et al (2006) found that granite eaten by broilers increased the gut health and empty weight of gizzard, while it didn't affect the growth performance significantly.

In the present experiment, broiler chickens were given either granite, zeolite, marble or non-grit to investigate the influence of different grit stones on performance of broilers especially in aspects of weight gain, feed conversion ratio, particle size

distribution of excreta and passage and disappearance of grit stones. This study focused on how did marble affect the performance of broilers.

2. Literature review

2.1 The digestion of broiler and the function of gizzard

Growth rate and feed conversion are extremely important for broiler production. With respect to this, a well-developed gizzard is essential to improve the profitability of broiler industry. The stomach of birds is composed of 2 compartments, the glandular stomach (proventriculus) where chemical digestion occurs and muscular stomach where the digesta are mixed and ground (Rougiere et al., 2012). As the birds eat, the ingested food is not ground but swallowed into the crop where the moisturization happens. The crop plays a role of transient storage room. It's observed that the feed enters directly either proventriculus or gizzard bypassing the crop when these two sections are empty. For the intermittent feeding, compared to ad libitum feeding, birds tend to store much more feed in the crop (Svihus 2014). After moisturization, the ingesta moistened goes further down to proventriculus where the secretion of hydrochloric acid and pepsinogen takes place. The retention time in the proventriculus is pretty short and the mixture of digesta, water, HCl and enzymes are thoroughly mixed and ground in the gizzard. As contraction of the gizzard muscle begins, a reflux of digesta between proventriculus and gizzard happens that enhance both the chemical digestion by prolonging the contact time between digesta and enzymes and the grinding activity for large particles (Svihus 2014). In addition, some materials are pushed into duodenum for further digestion and absorption. This is general procedure of broiler digestion.

The gizzard consists of two thick muscles and two thin muscles. There is a koilin layer inside of the gizzard rubbing against the digesta into particles of certain size. The grinding activity in the gizzard initiates with the contraction of thin muscle. The pylorus opens and the peristaltic contraction in the duodenum starts. The thick muscle contracts once the duodenal contraction starts. This causes the gastric materials to be pushed into

the proventriculus together with some materials being pushed into duodenum. When the thick muscles relax, the contraction of proventriculus will push the materials back to the gizzard (Svihus 2011). It establishes a contraction circle in this segment of digestive tract.

The function of gizzard can be influenced by many factors. Svihus (2011) reported that structural components such as oat hulls and wood shaving stimulate the gizzard to be enlarged in size and improve the holding capacity. Amerah et al (2008) found coarse grinding diet resulted in a heavier gizzard and higher feed efficiency. Sacranie et al (2012) reported that the gizzard function was improved when broilers fed on diets diluted with hulls. Mash diets tended to have heavier gizzards than pellet diet due to the lack of the structural components like coarse particles (Amerah et al., 2007; Abdollahi et al., 2014). All the studies mentioned above revealed that structural components enhanced the stimulation of gizzard and thus improve the performance of birds.

2.2 Use of grit as a digestive aid

Grit is termed as hard and sharp materials by Bethke (1926). Gionfriddo (1994) also describe grit as stones and rock fragments ingested by birds. According their solubility in the digestive tract, the grits are sorted into two types including soluble grit and insoluble grit. The insoluble grit includes granite, zeolite, granite, feldspar and so forth and they were observed to have a longer retention time in the gizzard compared to soluble grit (Gionfriddo 1994). However, they will get polished and shaped after constantly mechanical grinding. Soluble grits such as limestone, marble, oyster shell and other calcareous grits are easy to get dissolved in the gastric acid especially when fed with small particles.

It is a natural behavior for birds to ingest grit stones. Gionfriddo and Best (1996) found that grits were found in the majority of gizzards from American birds. As it is widely spread, the main function of grit is adding grinding ability of the gizzard by facilitating the mechanical breakdown of digesta (Gionfriddo and Best 1999). The second function of grit use is the supplement of the minerals. Birds with high

requirements for calcium preferentially ingest calcareous grit to compensate for the deficiency of calcium in order to maintain their lives (Gionfriddo and Best 1999). In addition, it was assumed that the grits in the gizzard had a function of buoyancy control during diving for king penguin (Beaune 2009). The grit is not essential for birds to survive but it is rather important due to its mechanical function in gizzard and nutritional benefits (Gionfriddo and Best 1999). Numerous studies have concentrated on the effects of different grit use on performance of birds.

Fritz (1936) investigated the effect of granite on digestibility in the domestic fowl. In his trial, all-mash diet and field peas diet were adapted to compare the change of digestibility when birds were fed with granite. The author detected that the influence of granite tended to be greater when coarse feed was fed even though the improvement was slight.

Heuser and Norris (1946) tested the response when giving single comb white leghorn cockerel chicks calcite grit and granite in the starting ration. It turned out that heavier and larger gizzards were got and feed efficiency was improved when birds received granite, whereas granite did not influence the body weight when birds were given mash diet. This provided some evidence that the grit made more difference when coarse and hard food were ingested.

Scott and Heuser (1956) evaluated the effects of both insoluble and soluble grit on the performance on broilers, turkeys and layers. The author concluded that insoluble grit improved the feed efficiency, egg production and growth compared with soluble group and no-grit group. Moreover, birds had a preference on feldspar to granite.

Norris et al (1974) examined the regulation of grit in the gizzard of Norwegian willow ptarmigan. The results showed that the grit present in the gizzard had connection with the seasonal availability, particle size and the food eaten by ptarmigan.

2.3 Marble and its effects in digestive system

Marble is a metamorphic limestone which is dominantly composed of calcite (CaCO_3) and little amount of dolomite ($\text{CaMg}(\text{CO}_3)_2$) (Segadães 2005). The physical and chemical properties are showed below (Table 1).

Table 1: Properties of marble from the marble supplier Visnes Kalk AS.

Physical and chemical properties of marble	
Appearance	Chips
Colour	White, greyish
Odour	Odourless
Solubility	Soluble in dilute acids. Water solubility: 0,014 g/l (20 °C), 0,018 g/l (75 °C)
Density	2.7 g/cm ³
pH in solution	8.5-9.5 (10% water solution)

The marble is easily dissolved when it reacts with gastric acid and hence increases the pH in the gizzard. Walk (2012a) suggested that limestone was approximately 80% dissolved in the gastrointestinal tract. The pH of gizzard content was measured to be between 4 and 5 when layer hens were given a diet of high calcium carbonate content (Svihus 2011). Simultaneously, the Ca content in the gastrointestinal tract increases dramatically when birds have free access to marble. These are two main impacts by feeding the marble to the broilers except for the grinding ability of gizzards.

The optimal pH value was reported to at around 2.8 for the chicken pepsin activity (Bohak 1969). As the calcium carbonate reacted with the gastric HCl, the pH in the gizzard increased significantly. An unfavorable pH would decrease the Ca solubility in the digestive tract and reduce the digestibility of protein (Walk et al., 2012b).

Calcium is of critical importance to birds in a physiological way, especially for the young birds and hens (Harper 1963). Dietary calcium (Ca) concentration has a great influence on the digestibility of phosphorus (P) (Li et al., 2014). It was reported that apparent ileal digestible P increased when the dietary Ca supplement was reduced (Tamim 2004.; Plumstead et al., 2008; Abdollahi 2015). The detrimental effects resulted from the reductions in endogenous phytase activity and calcium phosphate precipitation (Walk et al 2012a). The feed cost and P excretion decreases when improving the

utilization of P therefore it is necessary to control the amount of Ca ingested by broilers.

In addition to Ca, a certain amount of magnesium is found in marble. Arnon and Mehring (1964) observed that the dietary level of magnesium had no adverse effects on the performance of chicks when it was below 4000 p.p.m. Nevertheless, excessive intake of Mg resulted in that the excreta was not well formed. It was reported that feeding 1.2% Mg from magnesium carbonate to hens resulted in the decrease of egg production, feed efficiency, body weight of hens (McWard 1967). The marble used in this trial has low content of Mg.

3. Material and Methods

The experiment was conducted from 12th of November to 4th of December 2015 at the Center for Animal Experiments at Ås Gård in Ås. All laboratory work was done at the Department of Animal and Aquaculture Science at the Norwegian University of Life Sciences. All the work of this study were performed in collaboration with five other master students, Aorihan, Kari Borg, Biemujiafu Fuerjiafu, Cecilia Larsson, and Sodbilig Wuryanghai. Thus, the material and method have been written in cooperation.

3.1 Animal housing

252 day-old male broiler chickens (Ross 308) were randomly placed into four equal sized pens (72cm x145cm). The floors were covered with a thick layer of wood shavings. The birds had access to both feed and water ad libitum. Room temperature the first week was approximately 28 °C. Extra heating was provided by heat lamps over the pens the first 5 days to ensure that the chickens were in their thermos- neutral zone (approx. 30 °C). Room temperature was reduced down to 22 °C over the three following weeks. At 5 days of age birds were moved from the pens to quail cages (d. 35cm x w.50cm x h.20cm); 4 birds from one pen where randomly selected and placed in one quail cage, this was repeated 12 times for each pen, giving in total 4 birds x 12 replicates x 4 treatments = 192 birds divided on the 48 quail cages. Birds below 130 grams were excluded from the experiment. The extra birds were left in their pens, and

did not participate further on the experiment. The birds were exposed to continuous lightning due to no possibility for complete darkness.

The quail cages were equipped with both a feeder and a water container, and trays under to collect excreta. The quail cages were organised in two sections. Each side of each section contained 3 rows with four cages. The treatments were distributed among rows, and the patterns changed for each side of the sections.

3.2 Grit Stones

3.2.1 Granite

The granite grits were ordered from Sibelco Nordic AB, a supplier of industrial minerals. The grit stones were produced at Woldstad Sandforreting in Norway, and had a dimension of 2.0 to 3.5 mm.

The chemical composition of the grit stones is shown in table 2.

Table 2: Average values for the chemical composition of granite stones (Sibelco Nordic Sibelco n.d.)

SiO ₂	Silicon dioxide	79.50 %
Al ₂ O ₃	Aluminium oxide	9.57%
K ₂ O	Potassium oxide	3.62%
Na ₂ O	Sodium oxide	2.55%
Fe ₂ O ₃	Iron (III) oxide	2.04%
CaO	Calcium oxide	1.66%
MgO	Magnesium oxide	0.67%
TiO ₂	Titanium dioxide	0.28%

3.2.2 Zeolite

The zeolite with 1mm to 2.5mm dimension were ordered from ZEOCEM, AS. The chemical composition of the zeolite was provided in table 3 by EL spol. Sr.o. Division of laboratory service on 11.01.2016. The lab analysed 34 types of different chemical composition and only main elements are shared here.

Table 3: Average values for chemical composition of zeolite grit (ZEOCEM 2016).

SiO ₂	Silicon dioxide	68.54%
Al ₂ O ₃	Aluminium oxide	12.82%
TiO ₂	Titanium dioxide	0.166%
Fe ₂ O ₃	Iron(III)oxide	1.51%
CaO	Calcium oxide	3.32%
MgO	Magnesium oxide	1.13%
MnO	Manganosite	0.027%
P ₂ O ₅	Phosphorus pentoxide	<0.05%
Na ₂ O	Sodium oxide	1.351%
K ₂ O	Potassium oxide	2.93%
Ba	Barium	0.061%
Sr	Strontium	0.02%

3.2.3 Marble

The marble grit/gritstone was produced by Visnes Kalk AS in Lyngstad of Norway. The dimension of the gritstone was 0.5-2 mm. The chemical composition of the gritstones is shown in table 4. The chemical name of the gritstone is calcium carbonate (CaCO₃)

Table 4: Average values for chemical composition of zeolite grit (ZEOCEM 2016).

CaCO ₃	Calcium carbonate	98%
MgCO ₃	Magnesium carbonate	1%
Fe ₂ O ₃	Iron(III)oxide	0.1%
SiO ₂	Silica (granite)	0.6%

3.3 Experimental plan

The experiment can roughly be divided into three main parts, where the effect of different types of grit on a diet without whole wheat, interaction grit x whole wheat and particle flow were examined.

Diet and grit stone inclusion

Commercial diets were bought from the Norwegian feed company Norgesfôr. The whole wheat was supplied by Felleskjøpet. The birds had access to both feed and water ad libitum throughout the experiment time, with exception of the period when the effect of whole wheat and passage rate were examined. The birds were fed starter diet from day 0-11, grower diet from day 11-18. From 18-22 days of age the remaining birds got access to a mixed diet consisting of 15% whole wheat and 85% starter diet, except on day 21, when half the birds were given 50 g of whole wheat and the remaining birds were given 50 g of the grower diet. Coccidostats were included in the diet.

Grit stones were given to their respectively treatment group on day 5 (2 g/bird), 7 (3.75 g/bird), 9 (3.75 g/bird) and 18 (1 g/bird), 19 (1 g/bird) and 20 (1 g/bird). Grit stones were given on top of the feed. When diet was changed, the feed residues were saved for collecting grit stone residue. Therefore, one bird was given a total of 9.5 g/cage until 13 days of age before all remaining grit stone residues was removed. Bird weight were registered at 5, 11, 13, 18 and 21 days of age.

The feed consumption was measured at the same time, starting from day 5-11. Quantitative sampling of excreta was conducted from 5-11, 11-13, 13-18 and 18-21 days of age. These samples were frozen immediately for further analysis.

Dissection and starvation

One randomly selected bird from each cage was killed with a cranial blow followed by a cervical dislocation and dissected on day 13, 18, 21 and 22. The body weight of the dead bird was recorded. Full and empty gizzard weight was recorded on all dissection days. The crop was collected on day 21 and 22. Both gizzard content and intestines were frozen immediately for further analysis.

At day 20 feed was taken away at 21:00 and the birds were starved to 07:00 on day 21, where feed was again provided. On day 21, 1 bird was removed from each quail cage, marked with its cage number, and placed in a pen corresponding to its treatment with access to water and feed. The excreta trays were removed and cleaned. The birds had access to the feed for 5 hours, and the excreta trays were placed back after two

hours of access to feed to collect excreta. The trays were left to collect excreta produced during the following 3 hours. After 5 hours, the bird was killed with a cranial blow followed by cervical dislocation and dissected. After dissection of all 48 birds, the birds in the pens were placed back into their respective quail cage and given access to feed and water. At day 21 feed was taken away at 21:00 and the birds were starved to 07:00 on day 22. On day 22, the birds were given access to the feed for only 30 minutes. Two birds from each treatment were killed with a cranial blow followed by a cervical dislocation, 60, 90, 120, 150, 180 and 210 minutes after commencement of feeding.

3.4 Laboratory work

All the samples were first thawed then homogenized, respectively.

Dry matter:

Dry matter of feed, faeces, gizzard content, crop content, duodenum + jejunum content and ileum content were all determined with the procedure below:

A representative sample was taken out, wet weight registered, and then dried in an oven at $105 \pm 2^\circ\text{C}$ overnight. The sample was placed in a desiccator until cool the dry weight was measured. Tare weight of crucible was subtracted from the gross weight of the sample to calculate net weight of wet/dry sample (equation 1).

After measured dry matter content of each digestive tract segment and faeces from day 21, intact whole-wheat were picked out manually. To achieve this, the samples were diluted with water over night. The whole wheat was then dried again to find dry matter content. This was only done for the birds that were given access to whole wheat for two hours.

$$\frac{\text{net weight of dry sample (g)}}{\text{net weight of wet sample (g)}} \times 100\% = \text{Dry matter (\%)} \quad (1)$$

AME

Apparent metabolizable energy (AME) of faeces from 13-18 and 18-21 days of age were performed by lab assistant Frank Sundby according to NMBU's procedure; a representative sample of the homogenized faeces were dried overnight ($105 \pm 2^\circ\text{C}$) and

put in a bomb calorimeter (PARR 6400 Bomb Calorimeter) and values were calculated for each sample.

Gizzard pH:

Before the dry matter was determined in the gizzard content, pH was measured by VWR pH100 Which is produced by VWR company, with accuracy of $\pm 0.1\% \pm 2$ digit and resolution of 0.01 unit.

Separation of gritstones from in the gizzard and faeces

Due to a relatively small amount gizzard content, the whole sample had to be used for dry matter determination. Thus, the particles had to be dissolved in water before the using the floating method. The method consisted of holding the bowl under a slow running faucet with water rinsing through at a steady pace distributing the particles. As a result, the low density particles float up and were washed out, while the high density particles, the gritstones, are left in the bottom of the bowl. The grit stones were then dried in room temperature overnight and weighed the following day, and saved for further analysis.

The same process was used for faeces collected from 5-11 days of age. The faeces from each cage was homogenized, and a 250g sample were soaked in enough water to dissolve the particles. For faeces samples collected on 11-13, 13-18 and 18-21 days of age, the amount of grit stones were collected with the wet sieving procedure, as described below.

Wet sieving procedure

Wet sieving of faeces was done to determine the particle distribution on dry matter basis. Faeces from 11-13, 13-18 and 18-21 days of ages were first homogenized and analysed for dry matter content. According to the Standard Wet Sieving Analysis Procedure from The Centre of Feed Technology/Fôrtek at NMBU (Miladinovic 2009), the samples should have been dried in the sieves for minimum 4 hours to determine the dry matter, but due to practicalities and limited time, an alternative method was created

to determine dry matter of the particle distribution.

100 grams of sample were dissolved in water for 10 minutes with the assistance of a magnet stirrer (IKA C MAG HS7) before wet sieved in a Retsch sieve shaker (AS 200 Control) with amplitude 1.50 mm/g. Some additional water was used to rinse out the container with the sample to make sure all the particles were emptied into the sieves. Sieves size were 1.4, 0.8, 0.5 and 0.2 mm, and water pressure was at maximum. Sieving time were set to 2 min. with water, and 1 min. without water to shake off excess water. Each sieve was then weighed. Form 4 replicas per treatment for all sample sets, a sample of approximately 2.5 grams were taken out to determine dry matter of respective particle size in the sieve. The average dry matter content was further used to calculate the particle distribution of the faeces on dry matter basis. To estimate a “wet tare sieve weight”, empty sieves were shaken as mentioned and weighed. The average of 11 registrations was used when subtracting the tare weigh from the gross registration of the wet sample. The content left in the sieves were washed out in a bowl and rinsed for grit stones as described above, and the grit stones were collected and saved for further analysis.

Particle distribution of grit stones

Three representative samples from the original grit stones were dry sieved to find the actual particle size distribution of grit given to the birds. The tare of the sieve was first registered before about 100 grams of the initial grit stones were dry sieved for 1 minute on amplitude 1.00 mm/g on the Retsch sieve shaker (AS 200 Control), each sieve was then weighed and registered again before emptying the content of the sieves. All steps where repeated between each sample. Each type of grit stones were sieved 4 replicates to get an average particle distribution. Similar procedure was conducted for grit stones that were found in the faeces and gizzard. Since the samples of gritstones from the gizzard content was very small, the samples where pooled together from 12 replicas to 3 replicas so that the total sample were approximately evenly distributed within the treatments. Only zeolite and granite was detected in the gizzard content.

The percentage particle distribution was calculated with the equation shown

below.

$$\% \text{ of particle of nth Size} = \frac{\text{weight of sieve full (g)} - \text{weight of sieve empty (g)}}{\text{weight of sample (g)}} \times 100 \quad (2)$$

Due to human error, the particle distribution of initial grit stones were measured of the remaining grit stones in the bag, after the birds were fed. However, the particle distribution were assumed to be equal in the bag. A previous sieving had been done beforehand to get a quick picture of the actual particle size, but with a 500 g and no replicates. The differences in particles distribution will be discussed at the end.

3.5 Statistical analysis

Statistical analysis was performed by Professor Birger Svihus using a SAS software. The superscript a-b-c meant that different letters denoted significantly different difference ($p < 0.05$). Square root of measured square error was presented only when there were significant differences ($p < 0.05$).

4. Results

4.1 Growth performance

The weight gain and feed intake were recorded at 5, 11, 13, 18 and 21 days of age. FCR was calculated by formula $\text{FCR} = \text{Feed intake (g)} / \text{weight gain (g)}$. The weight gain of 4 treatments in different periods is shown in Figure 1. Weight gain per bird was significantly lower for the marble group ($p < 0.05$) compared to other groups in period 5-11, 5-21 and 5-18. No significant difference was found in period 11-13 and 18-21. In addition, weight gain per bird of marble group was significantly lower than zeolite group ($p < 0.05$) in period 13-18. In period 11-21, weight gain per bird of marble group was significant lower than granite and zeolite group ($p < 0.05$).

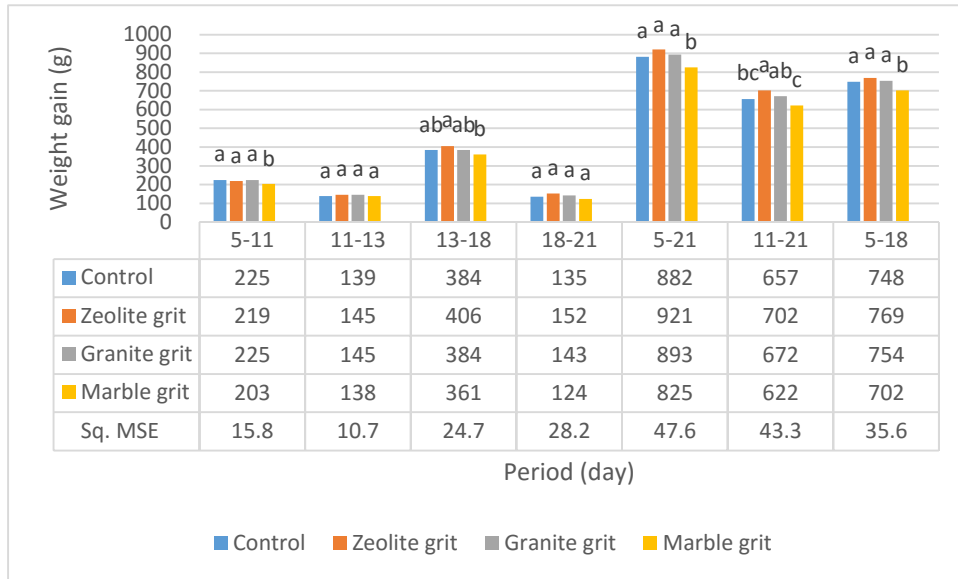


Figure 1: Weight gain of birds from 4 groups in periods of 5-11, 11-13, 13-18, 18-21, 5-21, 11-21, and 5-18 days of age.

a-b-c Means with different superscript are significantly different ($p < 0.05$)

Sq. MSE is square root of mean square error.

Feed intake per bird of 4 treatments in different periods is shown in Figure 2. The significant difference ($p < 0.05$) was found in 4 groups in period 5-11, 13-18, 5-21, 11-21 and 5-18, which the marble group had lower feed intake compared to other groups. The feed intake of birds from marble group was significantly lower than zeolite group ($p < 0.05$) in period 11-13. Moreover, feed intake of birds from marble group appeared to be significantly lower than granite and zeolite group ($p < 0.05$) in period 18-21.

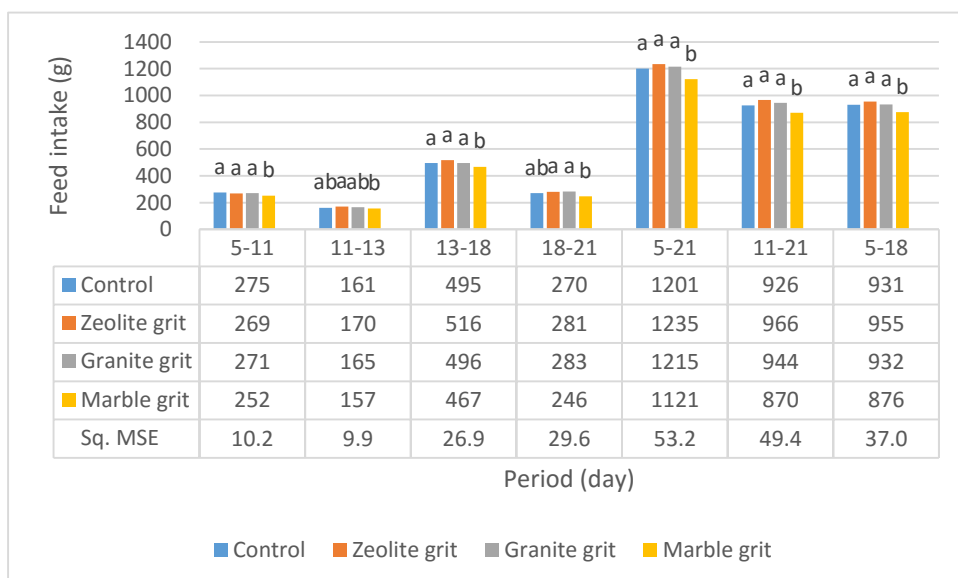


Figure 2: Feed intake of birds from 4 groups in periods of 5-11, 11-13, 13-18, 18-21, 5-21, 11-21, and 5-18.

a-b Means with different superscript are significantly different ($p < 0.05$)

Sq. MSE is square root of mean square error.

Feed / gain (g/g) of birds in four treatments is shown in Figure 3. No significant difference ($p > 0.05$) was found in any period among all the groups.

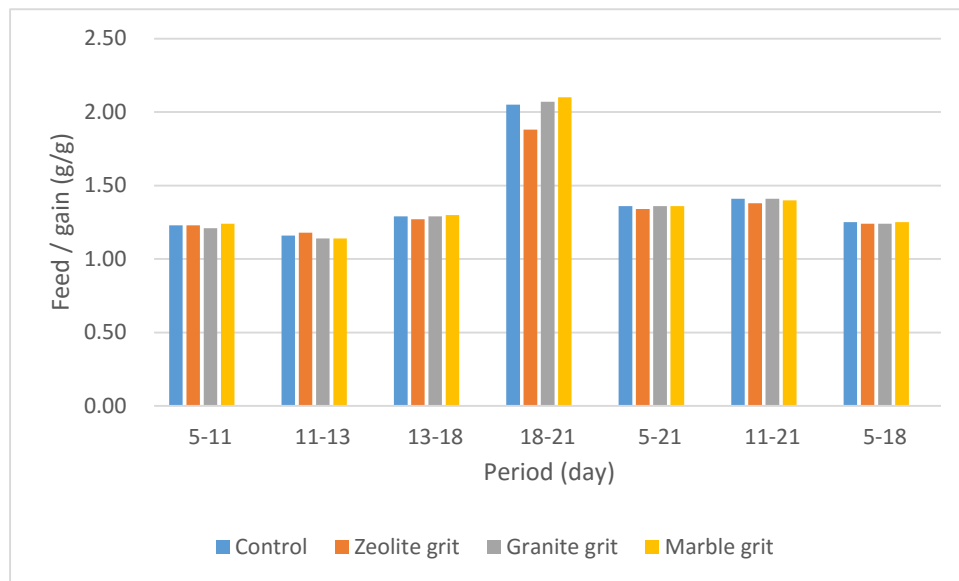


Figure 3: Feed / gain ratio of birds from 4 groups in in different periods of 5-11, 11-13, 13-18, 18-21, 5-21, 11-21, and 5-18.

No significant difference was found.

The weight of birds in four groups are recorded at 13, 18 and 21 days of age, which is shown in Figure 4. There was no significant difference ($p > 0.05$) between 4 groups.

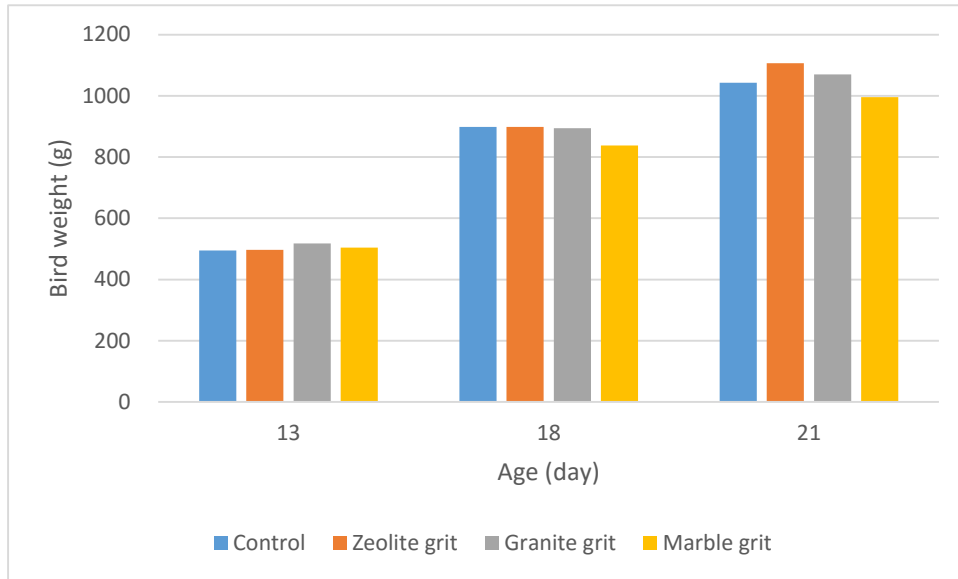


Figure 4: Bird weight at 13, 18 and 21 days of age

No significant difference was found.

4.2 Gizzard parameters

The pH of gizzard content of birds in 4 groups at the 13, 18 and 21 days is shown in Figure 5. The difference between 4 groups was not significant ($p > 0.05$) at any age.

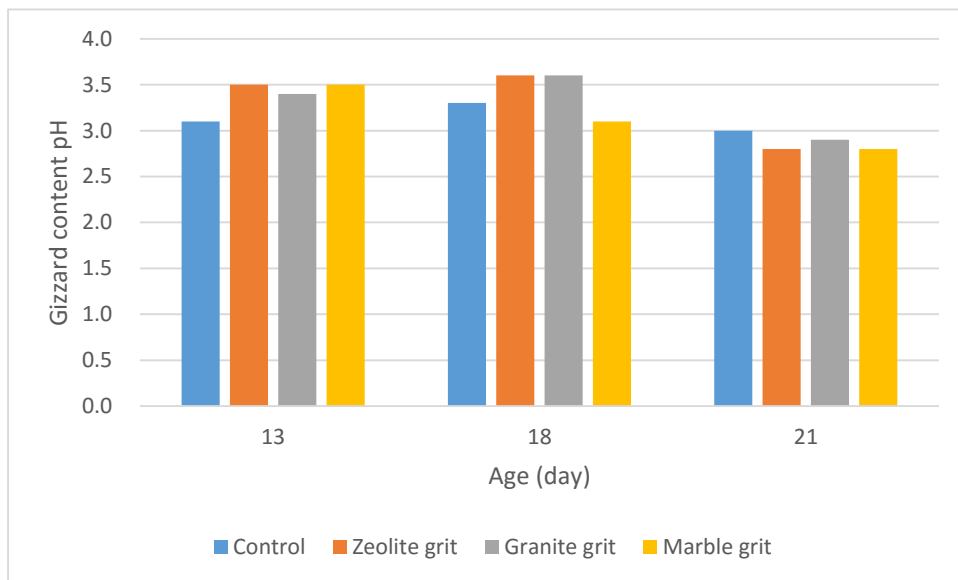


Figure 5: Gizzard content pH at 13, 18 and 21 days of age

No significant difference was found.

The gizzard content weight, relative gizzard content, empty gizzard weight and relative gizzard weight when birds were at 13, 18 and 21 days of age are shown in Figure

6, Figure 7 and Figure 8 respectively. Relative gizzard content was calculated by formula: $\text{relative gizzard content} = \frac{\text{gizzard content weight}}{\text{bird weight}} \times 100$. Relative gizzard weight = $\frac{\text{empty gizzard weight}}{\text{bird weight}} \times 100$. At 13 days of age, the marble group had a significantly higher value ($p < 0.05$) of relative gizzard content and gizzard content weight compared to granite group. It was also observed that the empty gizzard weight in marble group was significantly ($p < 0.05$) lower than granite group. At 18 days of age, no significant difference ($p > 0.05$) was observed from the parameters of gizzard. At 21 days of age, gizzards from marble group had significantly ($p < 0.05$) less content compared to gizzards from granite group.

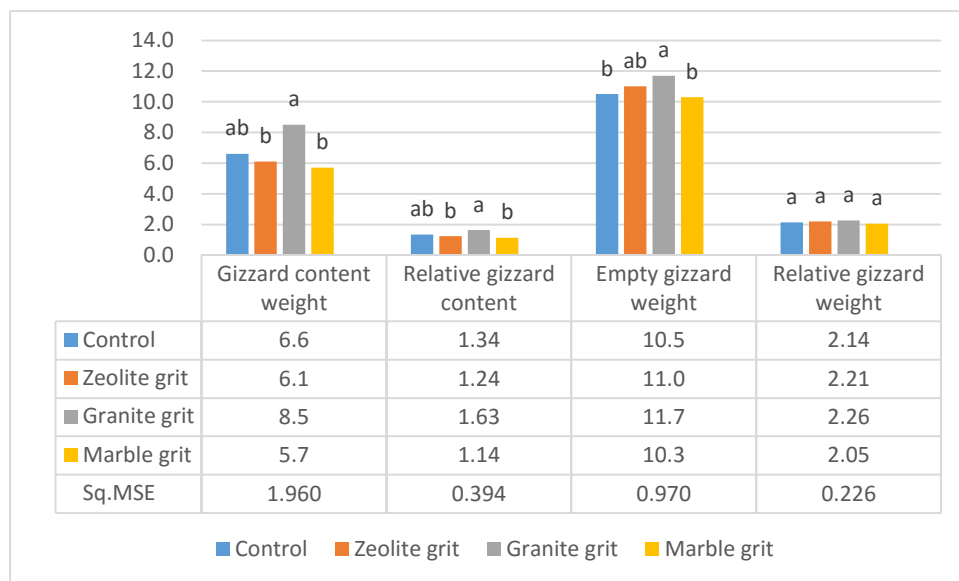


Figure 6: Gizzard parameters from day 13

^{a-b} Means with different superscript are significantly different ($p < 0.05$).

Sq. MSE is square root of mean square error.

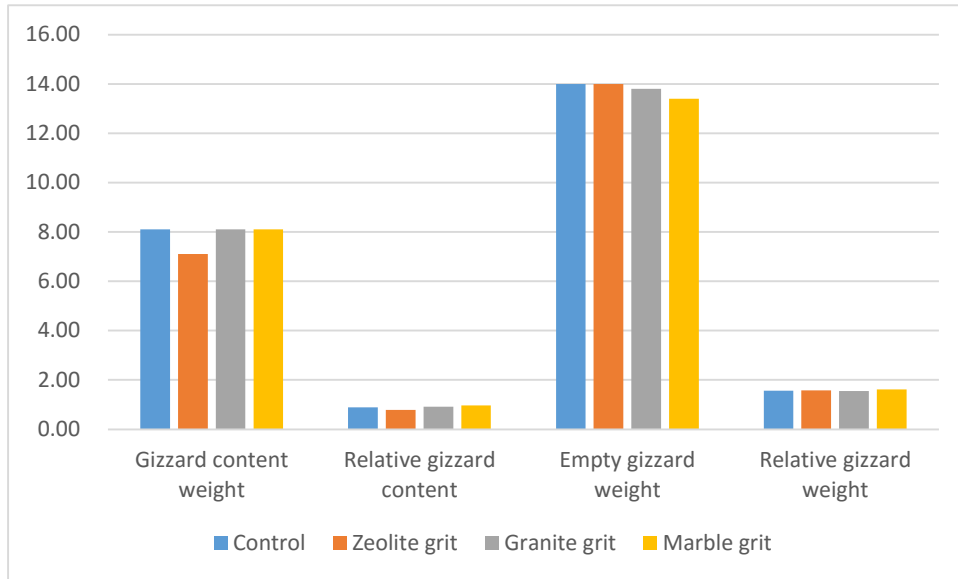


Figure 7: Gizzard parameters from day 18

No significant difference was found.

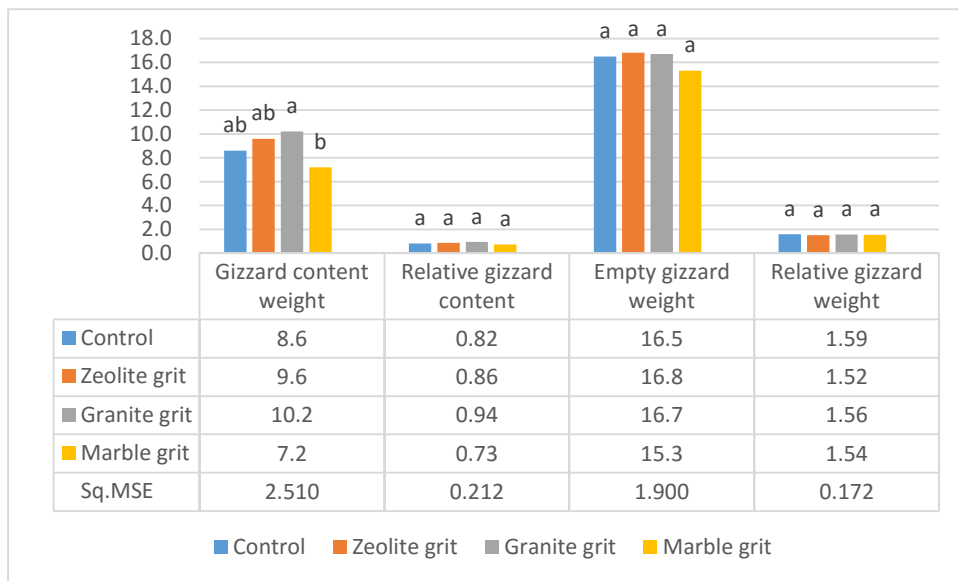


Figure 8: Gizzard parameters from day 21

a-b Means with different superscript are significantly different ($p < 0.05$).

Sq. MSE is square root of mean square error.

4.3 Particle size distribution of excreta

The excreta particle size distribution was recorded and shown in Figure 9 as well as Table 5 (excreta collected from day 11-13 period), Figure 10 (excreta collected from day 13-18 period) and Figure 11 as well as Table 6 (excreta collected from day

18-21 period). The extremely significant difference was found ($p < 0.001$) in the all the particle size level of excreta collected from day 11-13 period except for the particle size $> 1.4\text{mm}$. The marble group had a significantly ($p < 0.001$) lower proportion of particles (between 1.4mm and 0.8mm) compared to granite group and a significantly ($p < 0.05$) higher proportion of small particles ($< 0.2\text{mm}$) compared to granite and zeolite group. Moreover, marble group had a significantly ($p < 0.001$) lower proportion of particles (between 0.5mm and 0.2mm) and a significantly ($p < 0.001$) higher proportion of particles (between 0.8mm and 0.5mm) compared to zeolite group. Nevertheless, no significant difference ($p > 0.05$) was found in the excreta collected from day 13-18 period. For the samples collected from period day 18-21, granite group had a significantly higher proportion of excreta of which size is $1.4\text{-}0.8\text{ mm}$, and zeolite group had a significantly higher proportion of excreta of which size is $0.5\text{-}0.2\text{ mm}$.

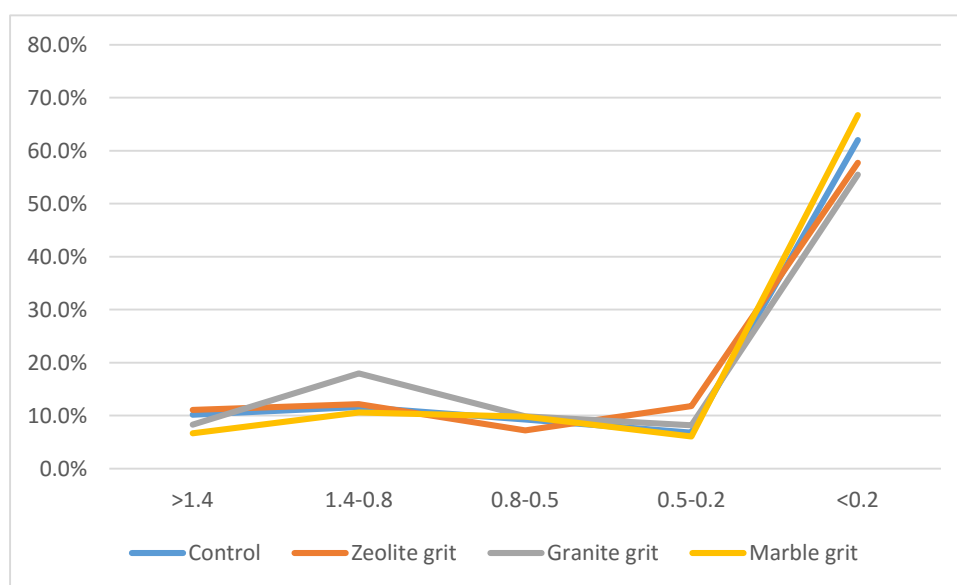


Figure 9: Excreta (collected from day 11-13) particle size distribution

Table 5: Excreta (collected from day 11-13) particle size distribution

Treatments Size (mm)	Control	Granite grit	Zeolite grit	Marble grit	Sq. MSE
>1.4	10.2% ^a	11.1% ^a	8.3% ^a	6.7% ^a	0.0460
1.4-0.8	11.6% ^b	12.2% ^b	18.0% ^a	10.6% ^b	0.0202
0.8-0.5	9.3% ^a	7.2% ^b	9.9% ^a	9.8% ^a	0.0148
0.5-0.2	6.8% ^b	11.8% ^a	8.2% ^b	6.1% ^b	0.0274
<0.2	62.0% ^{ab}	57.7% ^{bc}	55.5% ^c	66.7% ^a	0.0599

a-b-c Means with different superscript are significantly different ($p < 0.05$).

Sq. MSE is square root of mean square error.

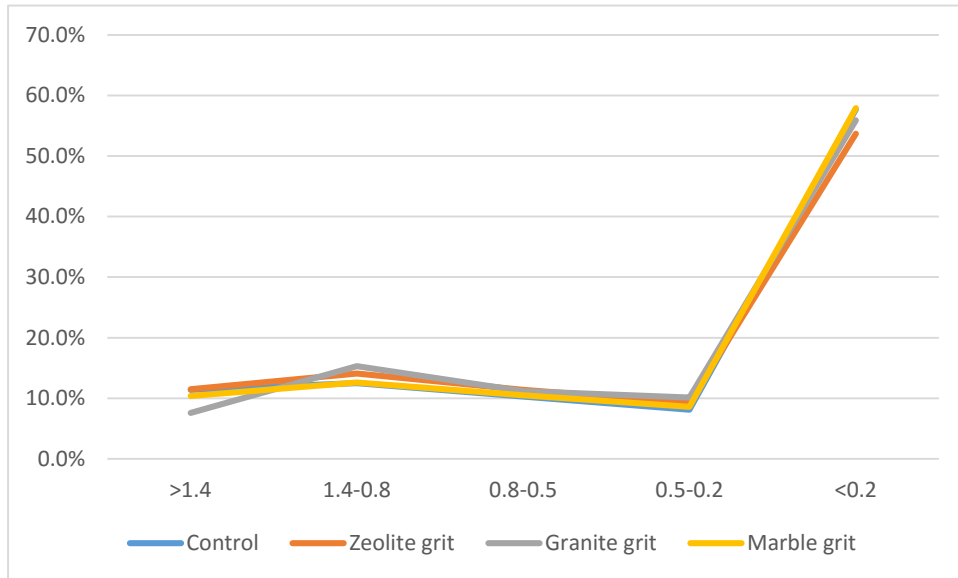


Figure 10: Excreta (collected from day 13-18) particle size distribution

No significant difference was found.

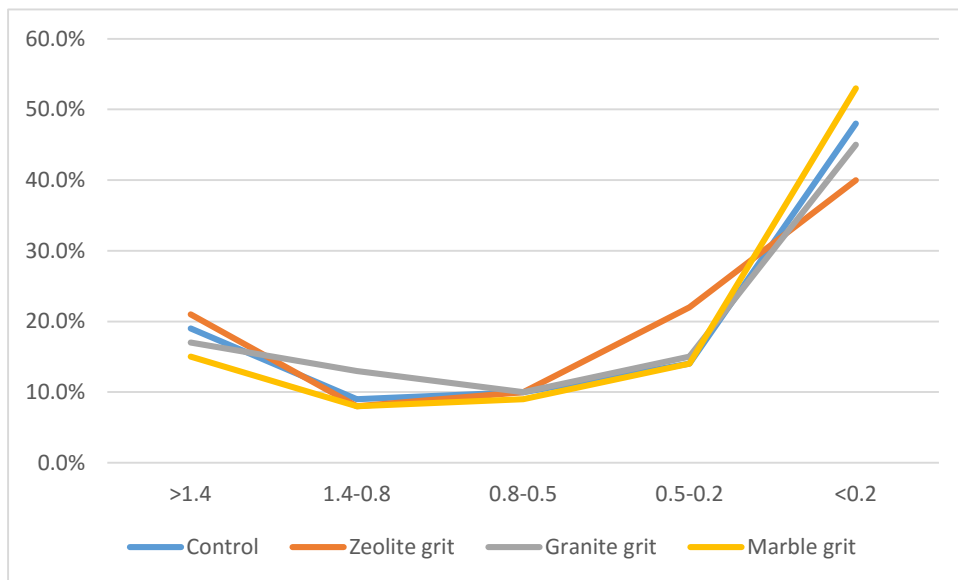


Figure 11: Excreta (collected from day 18-21) particle size distribution

Table 6: Excreta (collected from day 18-21) particle size distribution

Treatments Size (mm)	Control	Zeolite grit	Granite grit	Marble grit	Sq. MSE
>1.4	19% ^a	21% ^a	17% ^a	15% ^a	0.065
1.4-0.8	9% ^b	8% ^b	13% ^a	8% ^b	0.034
0.8-0.5	10% ^a	10% ^a	10% ^a	9% ^a	0.034
0.5-0.2	14% ^b	22% ^a	15% ^b	14% ^b	0.042
<0.2	48% ^a	40% ^a	45% ^a	53% ^a	0.145

a-b Means with different superscript are significantly different ($p < 0.05$).

Sq. MSE is square root of mean square error.

4.4 Grit eaten

The marble eaten by birds was significantly lower than granite and zeolite in period 5-12 ($p < 0.0001$), which is shown in Figure 11.

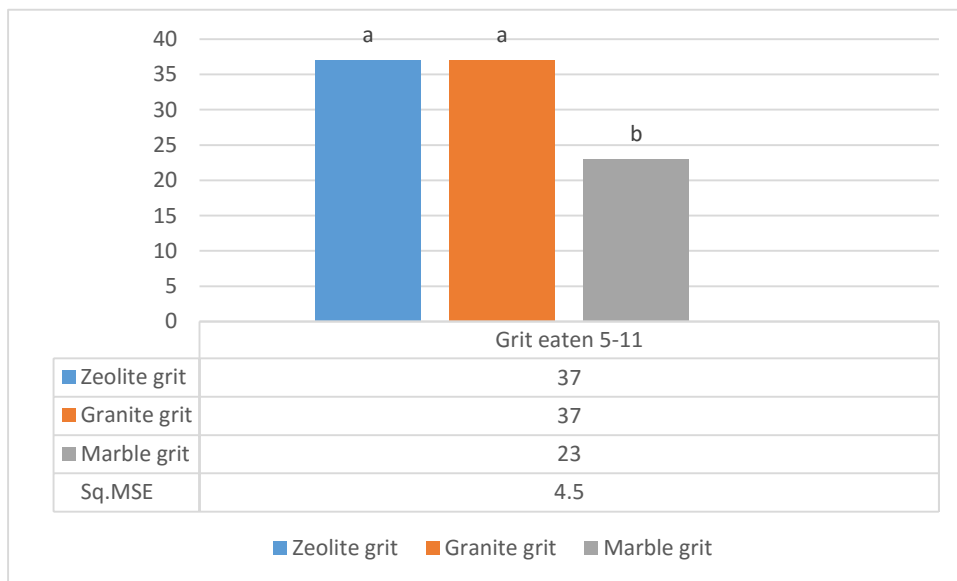


Figure 12: The amount of grit eaten by birds in period 5-11.

a-b Means with different superscript are significantly different ($p < 0.05$).

Sq. MSE is the square root of measured square error.

4.5 Grit passage

The grit passage and disappearance is shown in figure 13. In period 5-11 and 13-18, the passage of marble proportionally was significantly lower than granite and zeolite ($p < 0.0001$), whereas the passage of marble was significantly higher than granite

and zeolite ($p < 0.05$) in period 11-18.

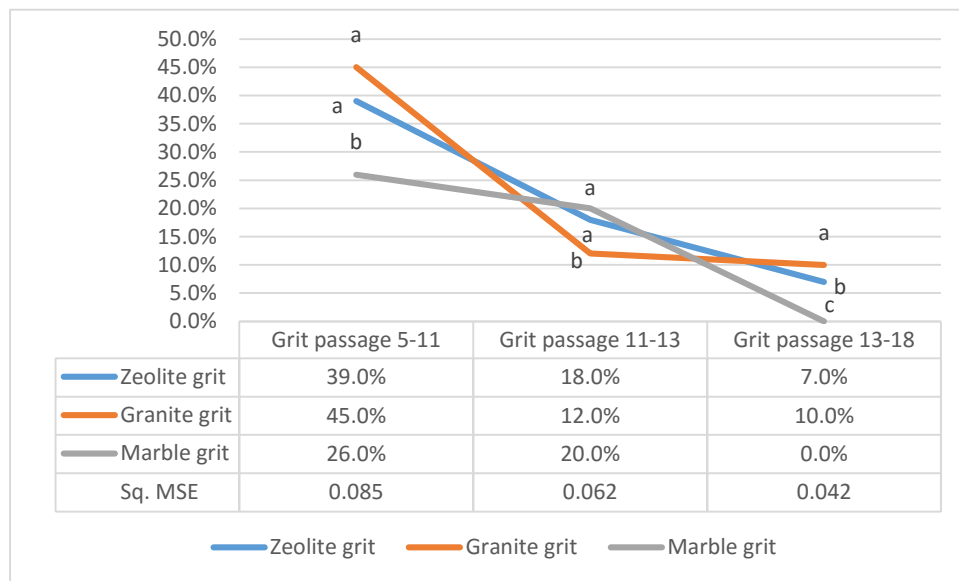


Figure 13: Grit passage in period 5-11, 11-13 and 13-18 and grit disappearance in period 5-18.

a-b-c Means with different superscript are significantly different ($p < 0.05$)

Sq. MSE is the square root of measured square error.

4.6 Grit disappearance

The marble disappearance was observed to be significantly higher than the disappearance of granite and zeolite ($p < 0.05$), which is shown in Figure 14.

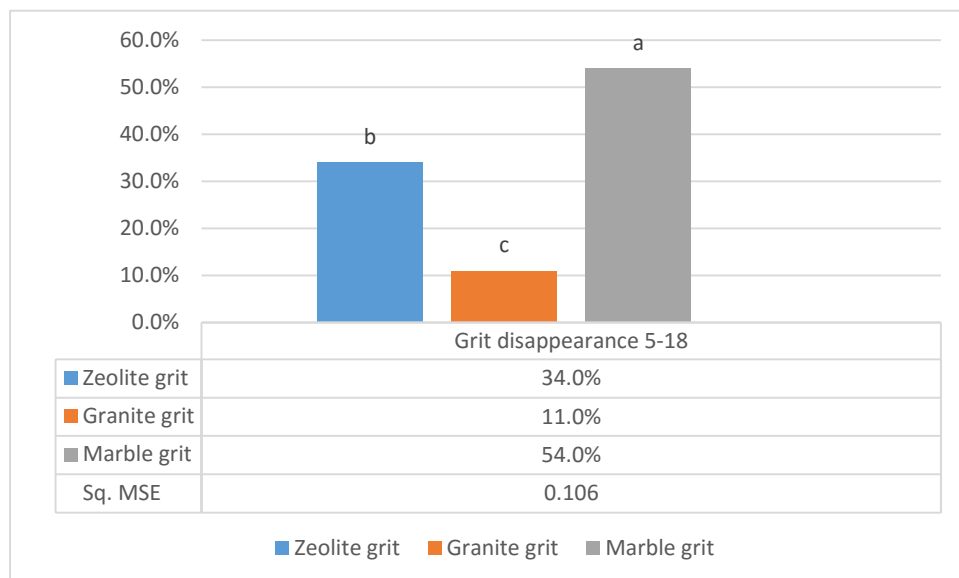


Figure 14: Disappearance of granite, zeolite and marble in period 5-18.

a-b Means with different superscript are significantly different ($p < 0.05$).

Sq. MSE is the square root of measured square error.

4.7 Particle size distribution of zeolite, granite and marble collected from excreta

Particle size distribution of zeolite, granite and marble in the excreta collected from period 11-13, 13-18 and 18-21 is shown in Figure 15, Figure 16, and Figure 17 respectively. Compared to original grit, the grits collected from excreta had a higher proportion of small particles and a lower proportion of large particles.

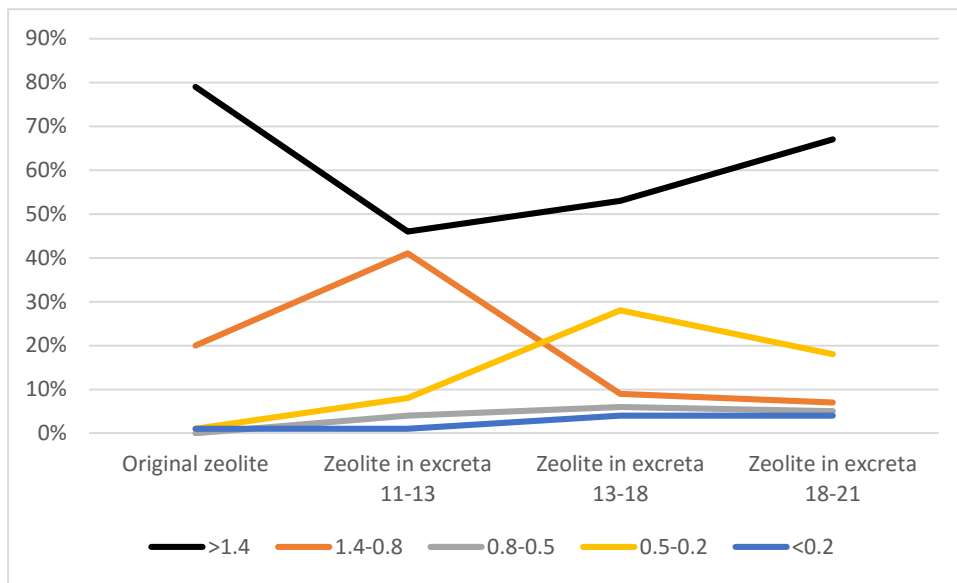


Figure 14: Particle size distribution of zeolite in excreta collected from period day 11-13, 13-18 and 18-21

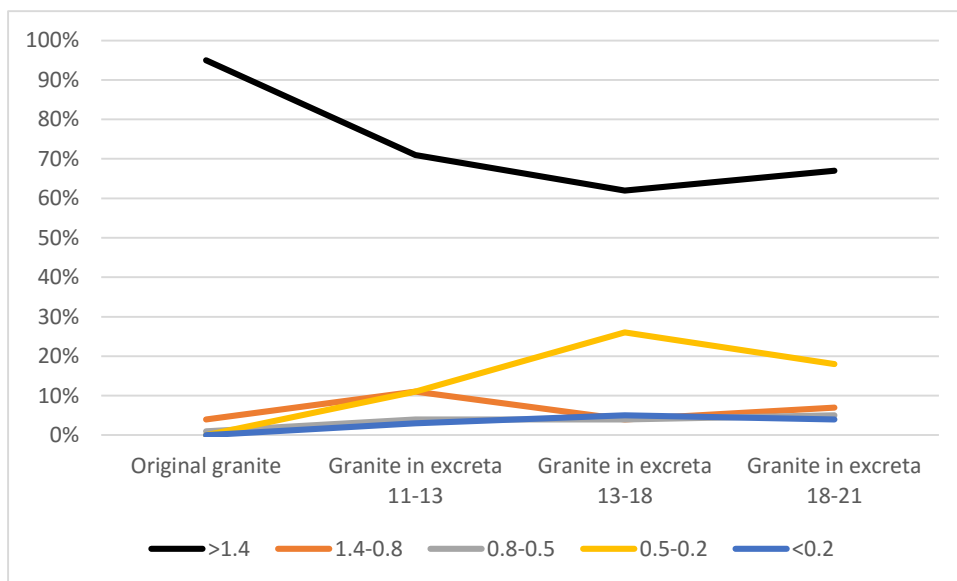


Figure 15: Particle size distribution of granite in excreta collected from period day 11-13,

13-18 and 18-21

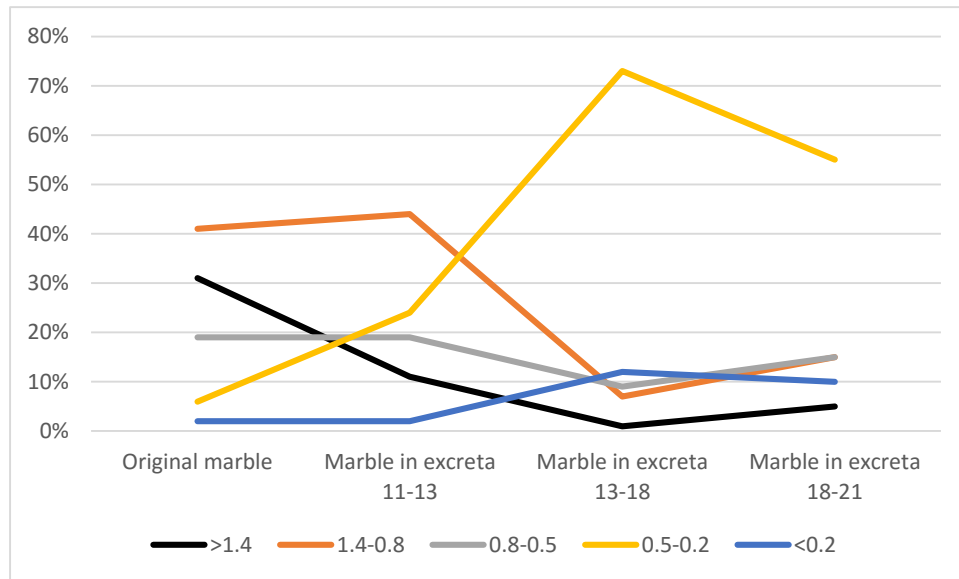


Figure 16: Particle size distribution of marble in excreta collected from period day 11-13, 13-18 and 18-21

4.8 AME (Apparent metabolizable energy)

The AME of four treatments is shown in Figure 17. There was no significant difference ($p > 0.05$) found in AME among 4 groups.

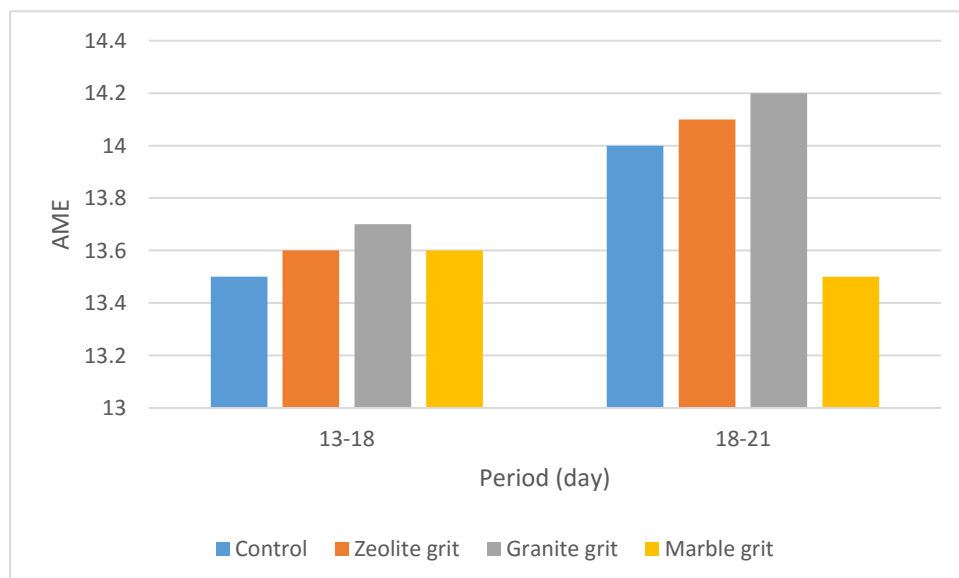


Figure 17: AME value of samples from period day 13-18 and 18-21

No significant difference was found.

5. Discussion

5.1 Growth performance of birds

Feed intake of birds was significantly lower with the supplementation of marble compared to other treatments in general during this trial. Insoluble grits were found to have no effects on feed intake in this study. The reason for the reduced feed intake with the marble supplementation can be logically explained by the chemical reaction between calcium carbonate from marble and hydrochloric acid secreted by proventriculus. The neutralization interfered the acidifying process in the digestive tract which slowed the progress of the digestion due to the deficiency of HCl and hence decreased the feed intake. Simultaneously, the rise in pH also had detrimental influence on the digestive efficiency through reduced enzyme activity (Walk et al., 2012). This finding confirmed the results from the study conducted by Hurwitz and Bornstein (1966), Hurwitz et al (1969) and Guinotte (1994), whereas results indicated by some previous studies were not in agreement with it. Taylor (1996) reported the conflicting results where no significant difference was found in feed intake between insoluble grit and soluble grit supplementation. The study conducted by Li et al (2014) indicated that the increase of Ca concentration by addition of limestone in starter feed did not affect the feed intake of the birds. Walk et al. (2012b) found that a low supplementation of dietary Ca did not influence the feed intake. Manangi and Coon (2007) had investigated the effects of different size of CaCO₃ on the birds' performance, and the optimal size of CaCO₃ to obtain the highest feed intake was reported to be 0.388 mm in his study, which was much smaller than the marble supplied in this study. The possible reason for the conflicting results might be that quantity and size of marble grit had influence on the feed intake of birds. The significant difference between marble group and control group was not found in later period when relatively smaller amount of marble was supplied. This might be attributed to the quantitative decrease of marble supplementation. When the marble supplementation decreased, the discrepancy between marble and control group was lessened.

A significant lower weight gain was observed in the period day 5-11 when a

relatively large amount marble was supplied. In general, marble supplementation impaired the weight gain of birds throughout the whole experiment. Lower feed intake was detected and stated above, which had great impacts on the weight gain. In addition, as mentioned in literature review, excessive Ca intake would decrease the digestibility of phosphorus and increased pH decreased the digestibility of Ca in the gut. The Ca level of the commercial diet supplied in this study was 0.92%. In order to utilize the phytate phosphorous, dietary phytase was supplemented into the diet to hydrolyze the phytate (Plumstead et al., 2008; Tamin and Angel, 2003; Manangi and Coon, 2008; Walk et al., 2012a). However, the hydrolysis of phytate was reduced due to the formation of insoluble Ca-phytate complexes (Plumstead et al., 2008) which also resulted in the unavailable absorption of Ca in the small intestine (Walk et al., 2012a). Furthermore, high dietary Ca was reported to lead to increased precipitation of Ca-inorganic soluble P which decreased the digestibility and absorption of P (Plumstead et al., 2008). Phosphorus was reported to account for approximately 30% of skeleton ash content (Li et al., 2014) and Ca is extremely important for maintaining bone mineralization (Abdollahi et al. 2015). The excessive of inorganic Ca had negative impacts on broilers during the growth period (Abdollahi et al., 2015). With the decrease of marble supplementation, the difference of weight gain between marble group and control group was not significant any longer. This was in accordance with the findings of Li et al (2014) and Walk et al (2012b).

Feed / gain ratio was not effected by neither insoluble supplementation nor marble supplementation in this study. Similar results were found by Taylor (1996), Garipoglu (2006), and Jones and Taylor (1999), whereas conflicting results were reported by Scott and Heuser (1956) and Smith and Macintyre (1959), where insoluble grit supplementation improved the feed efficiency of birds. It could be assumed that the granite and zeolite supplied were too small and too little to affect the feed efficiency of birds.

5.2 Effects of marble on stimulation of gizzard

In contrast to expectation, which was that birds consuming marble would gain

a higher pH value in gizzard content, there was no significant difference found in the pH of gizzard among four treatments in this study. The result was conflicting with that of previous studies conducted by Shafey et al (1991) and Guinotte et al (1995), where pH of digesta was increased by addition of limestone. There were some possibilities for the unexpected results. Firstly, a considerable amount of marble might get dissolved in the crop thereby increased the pH of crop. Hilmi et al (2007) reported that crop produced the organic acid and decreased the pH of crop. A higher pH in crop was found when layers fed calcium carbonate (Bolton 1965). Shafey et al (1991) also reported dietary limestone increased the pH of crop. According to Svihus (2014), the pH of gizzard decreases when structural components are supplied in diet, and this is due to that gizzard with larger volume obtained from stimulation of structural component allows for a prolonged retention time as well as more HCl secretion. The identical result wasn't obtained in this study because the supplementation of insoluble grits did not result in significantly different pH value. The second possibility was that, as found and mentioned in results section, higher feed intake was observed in control group and insoluble grit groups, which might lead to the lack of significant difference among the four treatments. The feed was neutral and increased the pH of gizzard content when ground and mixed with HCl. The extra feed intake compared to marble group increased the pH in accordance with the pH increased by reaction between marble and HCl generally. Thirdly, the amount of marble ingested by birds was relatively low and some of them might pass through the digestive tract shortly after. Simultaneously, birds made adjustments to the increase of pH and secrete more HCl for the digestion. For this reason, the ingestion of marble made little difference on the pH of gizzard content.

The gizzard content weight and empty gizzard weight of birds from marble group was not significantly different from control group, whereas the granite supplementation lead to significantly higher gizzard content weight, relative gizzard content and empty gizzard weight when birds were at 13 days of age. Abundant evidences showed that structural components in the diet increased the size and holding capacity of gizzard (Preston et al., 2000; Svihus 2011; Sacranie et al., 2012). The stimulative effects were due to the increased grinding ability by structural components

(Svihus 2011). According to Buckner and Martin (1922), the grinding efficacy of grit depended on the hardness and solubility. Insoluble grit with larger size and harder property was assumed to have longer retention time and more extensive grinding ability (Smith and Macintyre 1959). As expected, the size reduction of marble particle was observed. The determining factors of size reduction for marble were either abrasion caused by grinding activity or chemical dissolution by neutral reaction. These findings corresponded with what was anticipated theoretically. In addition, the significant difference between insoluble grits was also observed, where birds from granite group had higher gizzard content weight and relative gizzard content at 13 days of age. This could be explained by the harder property of granite grit and higher proportion of larger particles (>1.4 mm) supplied and retained in the gizzard compared to zeolite. The results were in agreements with what was found by Heuser and Norris (1946), Smith and Macintyre (1959), Jones and Taylor (1999) and Garipoglu et al (2006).

5.3 Particle size distribution of excreta

There was no significant difference found between marble group and control group in particle size distribution of excreta, whereas granite group had a significantly higher proportion of larger particles of excreta (1.4 mm -0.8 mm) and a significantly lower proportion of smaller particles of excreta (< 0.2 mm) for the samples collected from day 11-13. Moreover, granite had a significantly higher proportion of particles (1.4 mm -0.8 mm), and zeolite had a significantly higher proportion of small particles (0.5 mm -0.2 mm). Overall, grit supplementation had no effects on the particle size distribution of excreta in the present study. The hypothesis was that grit supplementation lead to a higher proportion of smaller particles and a lower proportion of larger particles in excreta with respect to the enhanced grinding ability. Because of the acid binding ability of marble and relatively smaller particles, the finding regarding the marble group was not surprising. However, the finding achieved from zeolite and granite groups was conflicting with what was expected. No satisfactory explanation could be made herein. There were also no published studies investigating the effects of grit supplementation on particle size distribution of excreta.

5.4 Ingestion, disappearance and passage of marble

The quantity of marble ingested by bird was significantly lower than granite and zeolite. There were many possibilities for this result. According to Gionfriddo (1994), the shape, size, color and chemical composition of grit had effects on the amount of grit consumption. Simultaneously, when the birds consumed the harder and coarser feed, large particles of insoluble grit were needed to help with the grinding activity (Norris et al., 1975). The pellet diet might result in a high consumption of insoluble grit in this study. Jones and Taylor (1999) reported that birds ingested limestone to a calcium appetite rather than a grit appetite, and it was also reported that birds preferentially ingested the calcareous grit when they had high requirements of calcium and were supplied with calcium-deficient feed (Gionfriddo and Best 1999). The smaller amount of marble consumption suggested that when birds ingested enough marble to meet the nutritional requirement, they tended to diminish the intake of marble. This finding was in agreement with results from study of Abdollahi (2015), who reported that birds had a specific appetite for Ca consumption and regulated the Ca intake when they had a separate source of Ca supplied. In addition, a practical possibility might also affect the ingestion of marble. Compared to granite and zeolite, the marble had higher proportion of small particles which might be easy to fall down and covered by feed (grits were given on top of feed in this study), which affected the availability of marble supplementation, resulting in a relatively low consumption of marble grit.

The marble disappearance was observed to be significantly higher than the disappearance of granite and zeolite, while zeolite disappearance was significantly higher than that of granite. The finding was in agreement with what was anticipated. Physical abrasion from the intensive grinding activity and chemical dissolution from reaction between CaCO_3 and gastric acid resulted in high marble disappearance. Compared to zeolite, granite supplied contained more large particles with harder property, and this contributed to a lower disappearance of granite.

The passage of marble grit was significantly lower than insoluble grit proportionally in both period day 5-11 and day 13-18. However, the finding was

inconsistent because granite had the lowest proportion of grit passage in period 11-13. The trend of the grit passage for both insoluble grits and marble was consistently downward. This might be due to the decreased grit supplementation after day 9. According to Gionfriddo (1994), birds consumed grit and excreted a certain amount of grit when they had free access to grit, and when the grit supplementation was limited or removed, the birds would retain grit in the gizzard and reduce the elimination of grit. The granite had more grit passage in period 11-13, whereas it had less grit passage in period 13-18 compared to zeolite. Although the mechanism of grit retention in the gizzard was unknown, it was reported to be related to diet, grit accessibility, grit size and so forth (Gionfriddo 1994). There was no marble found neither from the excreta collected from period 13-18 nor from gizzards from day 18 and it might be assumed that the marble ingested were dissolved completely in the digestive tract.

5.5 AME

There was no significant difference found in AME among four treatments. The finding from present study was in agreement with the finding from the study of Abdollahi (2015). Nevertheless, conflicting result was found by Jones and Taylor (1999), where grit-fed broiler had an improvement in AME. The hypothesis was that grit supplementation improve the AME due to the increased mixing and grinding activity, however, the effects of insoluble grit supplementation on birds' performance was not conspicuous. Ad libitum feeding contributed to a great feed intake and low feed efficiency (Buyse et al., 1996), which might have an influence on detecting the grit's effects on AME. A further study should be established to investigate the effects of grit supplementation on AME, with feed regime switching from ad libitum to intermittently.

6. Conclusion

The result of the present study indicated that marble supplementation had detrimental effects on the feed intake and weight gain of broiler chicken, whereas feed efficiency, body weight, pH of gizzard content, size of gizzard, particle size distribution

of excreta and AME were not affected. The performance of birds was not improved by insoluble grit supplementation. Further studies should be established to investigate the effects of both insoluble grits and soluble grits supplementation on the performance of birds given intermittent feeding.

7. References

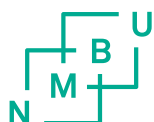
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