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Effect of Inclusion Zeolite as Grit in Commercial Pelleted Diet on Caged Broiler's Growth Performance, Gizzard Parameters and Excreta Size Distributions

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Table of Contents

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Abstract

The purpose of this experiment is to determine effects of inclusion zeolite as grit in modern caged broilers. A total of 252 day-old male broilers arrived in pens and were reared only commercial starter pellets and water until day 5. On day 5, starting point of experiment, 192 male chicks were randomly selected and assigned to 48 cages, each contain 4 chicks. Those birds in cages are equally divided in to four treatments and provided same type of diets all the time. Chicks in Control group (CG) only fed with diets without grit stone inclusion. Chicks in Zeolite group (ZG), Granite Group (GG) and Marble Group (MG) were separately provided zeolite grit, granite grit and marble grit, respectively.

The zeolite grit sizes 1~2.5mm were used in my experiment. A total of 12.5g/bird of each stone are provided on top of pellets by 6 different times, 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). The experiment measured particle distribution (PD) of excreta samples, stone PD in excreta; measured AME, growth performance including feed intake, weight gain and FCR; gizzard pH, gizzard weight, and gizzard content by different periods.

The result from end of experiment showed no significant difference birds weight gain, feed intake, feed utilization, gizzard size, gizzard pH and excreta among treatments. However, ZG had significant more weight gain at Day 11-21 and significant more in smaller size of excreta PD.

In conclusion, inclusion zeolite as grit with size range of 1-2.5mm have no negative effect on broiler growth performance and gizzard development but results in slightly higher weight gain.

Keywords: zeolite, broiler, weight gain, gizzard, excreta particle size distribution

1. Introduction

This thesis is written about the feeding trail which tested effect of zeolite as grit stone on caged broilers performance. The thesis consists of seven parts; Introduction, Background Information, Materials and Method, Results, Discussion, Conclusion and Possible Applications and References. The purpose of this experiment is to test the zeolite stone's effect on broilers, which describes in Background Information with relevant literature. The part, Materials and Method with an overview of experiment plan, explains the conditions in which we carried out and how we get those data as in Results. The Result part is figured out by zeolites effect on feed intake, gizzard parameters and growth performance. However, the Discussion part ends with bird's growth performance based on a different way of analyse. Followed by short Conclusion, the Possible Applications are shared as end of main content.

2. Background Information

2.1 Digestion in poultry

When we or other monogastric farm animals absorb nutrients in food or in feed, digestion begins before absorption (McDonald 2002). There are three types of digestion principles, mechanical digestion, chemical(enzymatic) digestion and microbial digestion (fermentation). Mechanical digestion is applying physical force, like mastication and stirring to breakdown particles; Chemical digestion means using endogenous enzymes to break down chemical bonds of large molecules. Fermentation is done by microbial enzymes to change big particles or form new nutrients.

There are two purposes. A, to reduce big feed particles or molecules down to many units which can be recognized and be accepted by absorption part of digestive tracts. B, to provide heat (including from friction, fermentation and forming new chemical bonds). Usually, those three different mechanisms are working together in each digestive tracts by different levels of efficiency. To maximize nutrients absorption, animal's different digestive tracts are developed to provide suitable conditions for different mechanisms. For example, in pigs, mastication in oral cavity contributes to majority mechanical digestion than mixing in stomach. Stomach and small intestine in pigs pooled enzymes into lumen to reach effective chemical digestion compared to that in pig's buccal cavity. Pig's hindgut keeps a majority of microorganism in the body to harvest unutilized nutrients which are escaped from the small intestine.

Comparing with a pig's digestive tracts, a chicken lacks teeth but has a crop, a gizzard and caeca. Thus, the journey of feed in bird's digestive tract is a different story. While a chicken picks feed into the buccal cavity, feed will be swallowed quickly due to absence of teeth to masticate and absence of moistened. After swallowing, feed slide down in lumen of the oesophagus and stored in a crop temporally in which feed can be moistened, softened and be fermented slightly. After leaving from a crop, feed is retained in a wider chamber, the proventriculus and is degraded. The enzymes or digestive juice released from proventriculus contributed to denature proteins in diets. Followed by proventriculus, a gizzard, a chamber equipped with big muscle layers can break down feed particles and mix with digestive juice. The feed is treated in gizzard is more likely with mastication process in buccal cavity in pigs. After feed pass through the gizzard, the fate of feed in chickens and pigs are similar.

However, mechanical digestion is the priority to maximize an efficiency of chemical and microbial digestion and it is important for better absorption. Thus, understanding form and function of chicken's stomach especially about proventriculus and gizzard is essential for poultry study.

2.2 Form and functions of proventriculus and gizzard in a chicken

Chickens are omnivores. One of the reasons that chickens are different from other omnivorous, is the anatomy of its digestive system. A chicken lacks teeth but has a beak, a crop and a stomach with two chambers; proventriculus (glandular part) and gizzard (muscular part). The proventriculus is connected from the oesophagus. The caudal chamber of proventriculus is connected with a gizzard by an intermediate zone which is a narrow junction lying between proventriculus and gizzard. The gizzard is connected to a cranial part of the duodenum by a very short narrow tube called pylorus (pyloric part). Appearance of bird's stomach is seemingly because of diets. In granivores and omnivores birds tend to have relatively small proventriculus than in the carnivores (Gionfriddo and Best 1999) . To digest feed, each part functions differently. The role of each part in digestion was sourced from its histology and its morphology.

Proventriculus is an open glandular chamber with four layers: the mucous membrane, submucosa, muscular tunic and serosa. The mucosa membrane secret mucus from the single layer of columnar cells to protect epithelium. The submucosa is a thin layer with nerve plexus. The muscular tunic has smooth muscles with two circular layers; the inner circular layer is well-developed and outer longitudinal layer is thinner. These muscles contribute to mix enzymes and feeds while rhythmic contraction.

The intermediate zone in Gallus (Calhoun 1954) is light-coloured constriction muscle layers with the elastic property but has no compound gland. This zone also has a relative smoothness surface which makes it distinguishable than other digestive sections. In Gallus, intermediate zone is variable in length due to eating habits of birds. Its length was 0.75 cm when Gallus has 4.5 cm long of proventriculus, as Hodges (1974) measured half a century ago.

In chicken, the proventriculus mainly has four functions; A. regulating temporary feed storage rate by coordinating with other organs; B. secreting digestive juice from the mucosa membrane to digest feed chemically; C. mixing digestive juice with feed by rhythmic contraction of the muscular tunic; D. limiting microorganisms by lowering pH. In this introduction, the first two functions of proventriculus are focused because they weight more than others in our study. About secretion of mucus, Chodnik (1947) described this secretion part in Gallus as mucous granules but 26 years later, Hodges (1974) and Horvath (1973) corrected as glands. The digestive juice from those glands is primarily composed of water, mucus, hydrochloric acid and pepsin. The number of glands will not be effected by size of proventriculus and diets but size of glands will influenced by diets (Rybicki and Lubanska 1959). Joyner and Kokas (1971) found that pH of gastric juice lowered to 2.6 in fasted chickens. While Gallus was fasting, those granules (glands) increased appearance and reached maximum accumulation after fasted 24 hours (Chodnik 1947). Once having a meal about 30 minutes later those granules started to reduce by evacuation, according to Chodnik (1947). He also found that when feed is *ad libitum,* 3 hours later the granules increases, and at around 6th hours achieves maximum numerous observation. About feed flow regulation, the contracted intermediate zone was narrowed as a barrier to separate the proventriculus and the gizzard. Under aiding of intermediate zone, proventriculus accepts a different amount of moistened feed from crop and digesta from gizzard.

Gallus gizzard is an open muscular chamber with four layers: Mucosa membrane, submucosa, muscle tunic and serosa. Those wall structure are connected from the intermediate zone, but appearance in gizzard is much thicker, which make functions differently.

Gizzard functions as mechanical breaking down, mixing and joining feed regulation. Gizzards in granivores, insectivores, herbivores and omnivores are shaped like a biconvex lens, which makes distinguishable from the gizzard in fish or meat eater bird in which gizzard is developed relatively round or sac-like structure, according to articles between 1872 to 1975 reported by King and McLelland (1981). Those two also mentioned that the shape in gizzard results from diets from different habitats. They also noted that biconvex lens-shaped gizzard has a thicker muscle tunic which break down hard feed items to increase a large surface area for acidic gastric juice. They also observed thickness of muscle tunic make gizzard varies in weight and pointed out that gizzard weight was an expression of the development of muscles, which we are using today.

Diets not only effect shape of gizzard but also have an effect on gizzard size or gizzard development in individuals. Birds peck various ingredients in the wild. To satisfy nutritional requirements, birds ingest grains, fruits, shells, bones, clays and even marble grits (Gionfriddo and Best 1999). Birds fed with those items, known as structural components, developed larger or heavier gizzards. The explanation for this is that diet structures will stimulate gizzard wall differently. To pass through the pylorus, harder and bigger size ingredients retain longer time than softer or smaller ingredients in order to be reduced to certain sizes. The longer time feeds retain, the larger gizzard is developed. Those ingredients are not alone to stimulate gizzard. Moreover, to maximize nutrient utilization from those feeds, birds peck indigestible items to aid gizzard's grinding further. The range of these items is large, from available wild sources like wood shavings, insect shells, feathers and different types of grit stones to artificial products like a lead shot (Gionfriddo and Best 1999). Those non-nutrient items usually have elastic property, filling effect and insoluble. Those could be swallowed by bird's voluntary or involuntary. Normally, those items are classified grit stones or by grit substitutes (Gionfriddo and Best 1999) .

2.3 Importance of grit and grit substitutes and its interaction with gizzards

Gionfriddo and Best (1999) defined grit as stones and rock fragments ingested by birds, excluding very fine particles such as dust, ash and clay. They also defined that grit substitutes are small hard items consumed by birds and seem to funtion as grit stones. Grit substitudes are various. Those could be hard seeds, insects parts, small snails and shell, shell fragments, fossils, bones, teeth, coral and even lead shot. Each grit stone or grit substitudes has its unique characteristic like colour, shape, size, hardness, solubility, chemical conpositions and structures. However, the purpose of bird's grit ingestion are mainly because of two reasons based on grit's function. A, grits or grit substitudes provide nutrients. B, grits can aid gizzards to grind better.

Grits and its substitutes provide nutrients because of its solubility and its chemical compositions (Gionfriddo and Best 1999). Grit substitutes like whole grains and seeds provide macro nutrients for bird's growth and shells or insoluble stones can release minerals which are essential, especially for laying birds in reproduction seasons. Thus, availability of and searching for those items can effect migration patterns on wild birds, including game birds (March and Sadleir 1970, March and Sadleir 1975).

Grits aid gizzard's grinding to break down hard ingredients and thus more surface area can be exposed to enzymes (McLELLAND 1979, Gionfriddo and Best 1999). To grind hard ingredients better, granivores consume significantly more grits than insectivores, frugivorous and omnivores (Gionfriddo and Best 1996). Studies in poultry also shown that not benefit for birds fed insoluble grits with fine particle diets or soft feed ingredients (Sibbald and Gowe 1977, Svihus, Herstad et al. 1997) but giving coarse diets with insoluble grits make chicken perform greater (Fritz 1937, Balloun and Phillips 1956, Smith and MacIntyre 1959, McIntosh, Slinger et al. 1962).

Besides those two explanations, birds consume grit for other reasons which is not clear. For example, gizzards in Graviidae, a diving species, has grits (King and McLelland 1981). They may ingest to increase density to dive deeper to scavenge.

However, studies also showed that when giving insoluble grits birds performed differently. Some results showed no improvements in birds performance (Fuller 1958, Sibbald and Gowe 1977, Bennett and Classen 2003). A recent study in turkey (Majewska, Mikulski et al. 2009) showed that insoluble grit has negative effect on bird's performance. These results are maybe due to lack of details of grits characteristics. Grit's hardness, durability and size ranges (Buckner and Martin 1922, Smith and MacIntyre 1959) are important and they are not same in each study. Thus, testing other stones with different hardness and durability may consider to carry out. Since a review from Shariatmadari (2008) reported zeolite powder has benefits on poultry gut health and performance, an attempt to test zeolite as stone form is set. In order to understand further performance of zeolite, its disappearance and passage are monitored.

2.4 Zeolites in poultry

Zeolites crystalline, hydrated aluminosilicates of alkali with alkaline cations. Clinoptilolite, as a common zeolites found in volcanic sediments, forms three dimensional structure from minerals at low temperature (Bernal and Lopez-Real 1993). The pure chemical elements of Clinoptilolite is Ca Na (AlO2)6 (SiO2)30 24H2O (Olver 1997). Pore structure as adsorbents and huge capacity in cation exchange without change it structure made its roles more divers in many areas (Bernal and Lopez-Real 1993, Martin-Kleiner, Flegar-Meštrić et al. 2001, Pappas, Zoidis et al. 2010) . In animal production, applying zeolite can improve feed utilization in the ruminants (Mumpton 1985) and in the monogastric (Papaioannou, Katsoulos et al. 2005, Suchý, Strakova et al. 2006).

However, applying zeolite in poultry (Evans, Singh et al. 2005, Shariatmadari 2008) is based on its two main functions; A. to lose and gain water reversibly. B. to exchange various cations selectively without change its structure. Majority study of it is to bind mycotoxins and to improve mineralization (Shariatmadari 2008). Few of those studies have used as grit stone.

In our feeding experiment, we use as grit stone with light green colour. Since it is volcanic sediments with pore structure, hardness ('1.5~2.5') and bulk density (1600–1800 kg/m3) of natural form (Rehakova, Čuvanová et al. 2004) are lower that hardness of granite ('7') which used to give birds as grit. The chemical composition of Clinoptilolite grit supplied to out experiments shows in **Table 1**.

2.5 Objective of this research

The current study tests zeolite as grit in order to evaluate:

1. Whether caged broilers fed with commercial pellets need zeolite as grit to perform better or not.
2. Whether effect of zeolite grit can provide beneficial indication or not.

3. Materials and Methods

The material and method part was written in cooperation with five other master students, Aorihan, Biemujiafu Fuerjiafu, Cecilia Larsson, Kari Borg and Huan Liu, because my topic is part of the comprehensive study which aimed to find the effect of different grit stones on modern broilers and interactions between whole wheat and those stones. The feeding experiment was conducted at Animal Production Experimental Centre (Ås Gård) of the Norwegian University of Life Science (NMBU), Ås, Norway, from 12th of November to 4th of December 2015. Lab work and statistics analysis were finished before April 2016.

3.1 Feeding Trail

Animals and Housing

In whole period, birds were housed on two places; day 1 to day 5 housed in pens and from day 5 until finished the experiment housed on cages. There were four equal sized pens (72cm x145cm x 50cm) covered with a thick wooden shaving layer. On each layer, birds had access to both feed and water ad *libitum*. To achieve both thermo-neutral zone and light for chicks, heat lamps and light was provided 24 hours per day in pens until day 5. Temperature was controlled by adjusting distance between layers and heating lamps. The quail cage (L. 35cm x W.50cm x H.20cm) were organised in 3 rows x 4 columns, divided on each side of two sections. The treatments were divided into rows, and the patterns changed for each side of the sections. Each cage was equipped with both a feeder on right side and a water container on left side and a removable tray was set under the cage to collect excreta.

A total of 252 day-old male broiler chickens (Ross 308) were randomly placed in a pen until day 5. On day 5, 192 birds above 130-gram weight were moved from the pens to cages randomly until day 22; 192 birds = 4 birds/cage * （48 cages. 48 cages = ）12 cages/treatment * 4 treatments.

Diets and Zeolite

In whole feeding experiment 4 types of diets and 3 types of grit stones were used; commercial pelleted starter, commercial pelleted grower diet, whole wheat and 15% whole wheat + 85% the starter diet. The both commercial pelleted starter and grower diets were from Norgesfôr, Norway, and whole wheat diet was from Felleskjøpet.

The zeolite with 1mm to 2.5mm dimension were ordered from ZEOCEM AS. The grit particle distribution was also mentioned in (lab work). The chemical composition (**Table 1)** of the zeolite was provided by EL spol. Sr.o. Division of laboratory Service on 11.01.2016. The lab result showed 34 types of different chemical composition and only main elements are shared here.

Table 1 : Average values for chemical composition of zeolite grit (ZEOCEM 2016).

SiO_2	Silicon dioxide	68.54%
Al_2O_3	Aluminium oxide	12.82%
TiO2	Titanium dioxide	0.166%
Fe_2O_3	Iron(III)oxide	1.51%
CaO	Calcium oxide	3.32%
MgO	Magnesium oxide	1.13%
MnO	Manganosite	0.027%
P_2O_5	Phosphorus pentoxide	<0.05%
Na_2O	Sodium oxide	1.351%
K_2O	Potassium oxide	2.93%
Ba	Barium	0.061%
Sr	Strontium	0.02%

Treatments

Based on objective, birds were arranged into 4 groups by with 3 different stones, granite, zeolite and marble separately supplied in each group and one group was without stones.

Starvations

The first starvation was planned to test interactions with whole wheat and grit. The second starvation was to determine feed flow.

First starvation lasted 10 hours, from day 20 at 21.00 to 07.00 on day 21. One bird was randomly marked and placed to pen. The remaining one had access to the feed for 5 hours and then killed. The excreta trays were placed back after two hours of access to feed to collect excreta for following 3 hours. The birds in pen was returned to responding cage to starve at 21.00 again.

Second starvation lasted 10 hours from day 21 at 21.00 to 07.00. At 07.00, the bird from each cage was given access to the feed for only 30 minutes. After getting access to feed exactly 60, 90, 120, 150, 180 and 210 minutes, two birds from each treatment were killed the same way as on day 21.

3.2 Data collection

Feed residues with uneaten grit and excreta

Feed residues with uneaten grit from a feeder was collected and then stored in sample bags for later lab work. Excreta on the tray was collected from each period 5-11, 11-13, 13-18 and 18-21 days of age. These samples were frozen immediately for further analysis.

Dissection

On day 13, 18, 21 and 22, one bird from each cage was randomly selected to be killed with a cranial blow followed by a cervical dislocation. After killed, body weight of each bird, the full and empty gizzard weight was recorded. The crop was collected on day 21 and 22. Both gizzard content and intestines were frozen immediately for further analysis.

3.3 Lab work

At the beginning of the lab work, all samples were thawed first and then homogenized.

Dry matter

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

A representative sample was taken out, wet weight registered, and then dried in an oven at 103 ± 2℃ over the night. The dried hot sample was placed in a desiccator until the sample is ambient before the dry weight is measured. Tare weight of crucible was subtracted from the gross weight of the sample to calculate net weight of the 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 overnight. The whole wheat was then dried again to find dry matter content. This manually separation only did for the birds that were given access to whole wheat for two hours.

(net weight of dry sample (g))/ (net weight of wet sample (g)) ×100%=Dry matter (%) (1)

Gizzard pH

Before the dry matter was determined in the gizzard content, pH was measured VMR pH measurement.

Separation of grit from gizzard content and faeces

Due to 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 use of floating method. The method consisted of holding the bowl under a slow running faucet with water rinsing through a steady pace distributing the particles. As a result, the low-density particles float up and 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 were weighed on 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 were homogenized. A 250 g 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 determined 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 measurements was used after subtracting the tare weight from the gross measurement of the wet sample. The content left in the sieves were washed out in a bowl and rinsed for grit stones as described above.

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 were repeated for each sample. Each type of grit stones was sieved four 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 slight, the samples were 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.

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

3.4 Statistics analysis

Data from experiment was subjected to one-way ANOVA using SAS software.

3.5 An Overview of Experiment Plan

The overview of the plan **(Table 2)** shows what actions we took on different days.

Table 2. An overview of plan

Period (day)	**1 to 5**	**5 to 11**			**11 to 13**	**13 to 18**	**18 to 21**			**21**	**22**
Diet	starter	starter			grower	grower	15%+85%			15%+85%	15%+85%
Day given grit		5	7	9			18	19	20		
Grit g/bird		2	3.75	3.75			1	1	1		
Sampling day		5			11	13	18			21	22
Samples and action		Register feed & Body weight			Feed residues, Excreta collection, Body weight	Dissection, Feed residues, Excreta collection, body weight	Dissection, Feed residues, Excreta collection, Body weight			Starvation, Feed residues, Excreta collection, Body weight, Dissection	Starvation, Feed residues, Excreta collection, Body weight, Dissection

4. Results

4.1 Broiler performance parameters

In broiler performance parameter, body weight, weight gain, feed intake and feed per gain (FCR) were compared among treatments by four different periods, Day 5-11, Day 11-13, Day 13-18 and Day 18-21.

Body weight

None of the treatment made a significant difference in body weight of birds at three different dissection days. Even though, body weight from ZG achieved 1107g, which was higher than 1043g, 1070g, 996g from CG, GG and MG, respectably.

Weight gain

In whole experiment period (Day 5-21), birds in ZG has not significantly higher weight gain than in CG and GG but the gain in ZG was significantly ($P<0.001$) greater than MG. From Day 5-11, ZG's weight gain has no significance than CG's and GG's but significantly higher than MG's. However, in the period (Day 11-21, the weight gain in ZG was significantly ($P<0.001$) higher than the gain in CG and MG.

Feed intake

No significant difference in feed intake among CG, GG, and ZG in all periods was observed. Feed intake in MG was significantly lower than other three groups in all different periods.

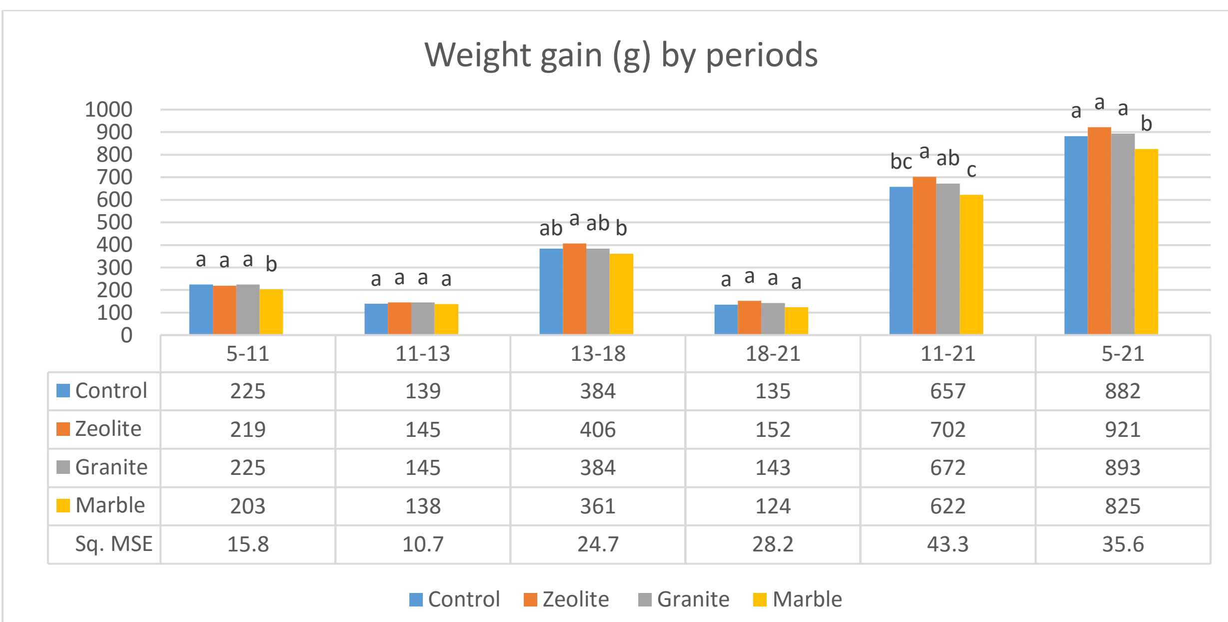
Feed Conversion Ratio (FCR)

There were no significant differences in FCR among all treatments in all different periods. FCR in Day 5-21 in ZG showed 1.34, which is lower than other treatments 1.36 but not significant.

Fig. 1: (**A**) Body weight (g) of birds on different dissection days

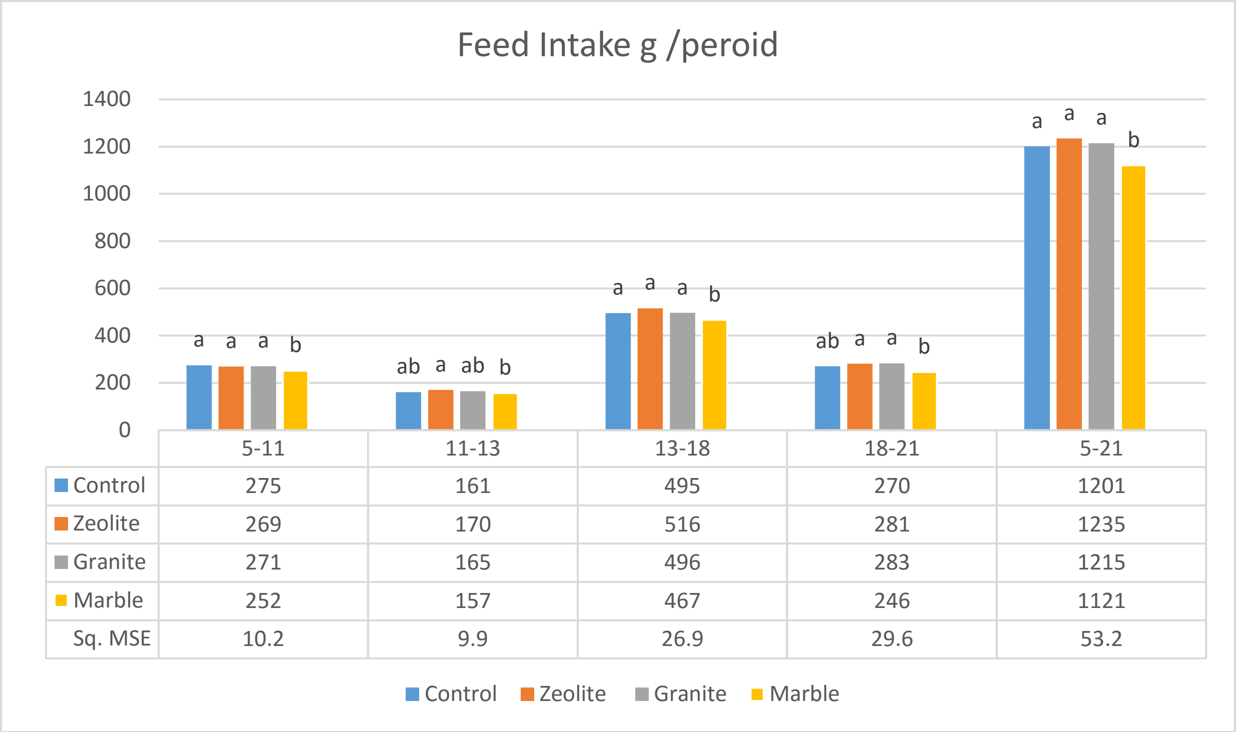
	Day 13	Day 18	Day 21
Control	495	898	1043
Zeolite grit	497	898	1107
Granite grit	518	894	1070
Marble grit	504	838	996
Sq. MSE	31,5	73	104,7

Fig. 1: (**B**) Weight gain (g) by periods.



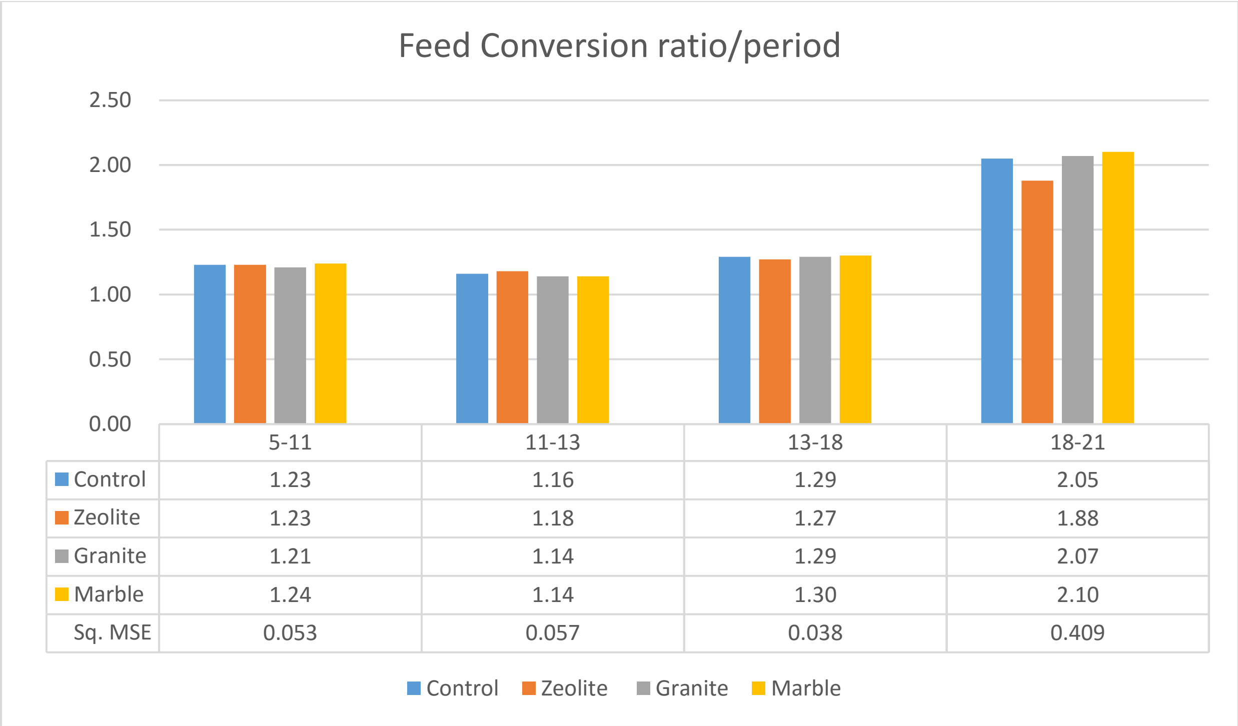
	5-11	11-13	13-18	18-21	11-21	5-21
Control	225	139	384	135	657	882
Zeolite	219	145	406	152	702	921
Granite	225	145	384	143	672	893
Marble	203	138	361	124	622	825
Sq. MSE	15.8	10.7	24.7	28.2	43.3	35.6

Fig. 2: (**A**) Feed intake (g) per period.



	5-11	11-13	13-18	18-21	5-21
Control	275	161	495	270	1201
Zeolite	269	170	516	281	1235
Granite	271	165	496	283	1215
Marble	252	157	467	246	1121
Sq. MSE	10.2	9.9	26.9	29.6	53.2

Fig. 2: (**B**) Feed per gain ratio by periods.



	5-11	11-13	13-18	18-21
Control	1.23	1.16	1.29	2.05
Zeolite	1.23	1.18	1.27	1.88
Granite	1.21	1.14	1.29	2.07
Marble	1.24	1.14	1.30	2.10
Sq. MSE	0.053	0.057	0.038	0.409

4.2 Gizzard parameters

Gizzard weight

Empty gizzard weight was recorded to have a comparison among treatments. There was no significant difference in empty gizzard weight among treatments in all dissection days.

Gizzard relative weight

Gizzard relative weight (Gizzard weight/body weight) did not display significant difference among treatments at each dissection day.

Gizzard content weight

Gizzard content weight from ZG at both day 13 and day 21 was not significantly different from the weight content from CG and GG, but it was significantly higher than the weight from MG at same days. However, on day 18 there was no significant difference in weight content of gizzard among all treatments.

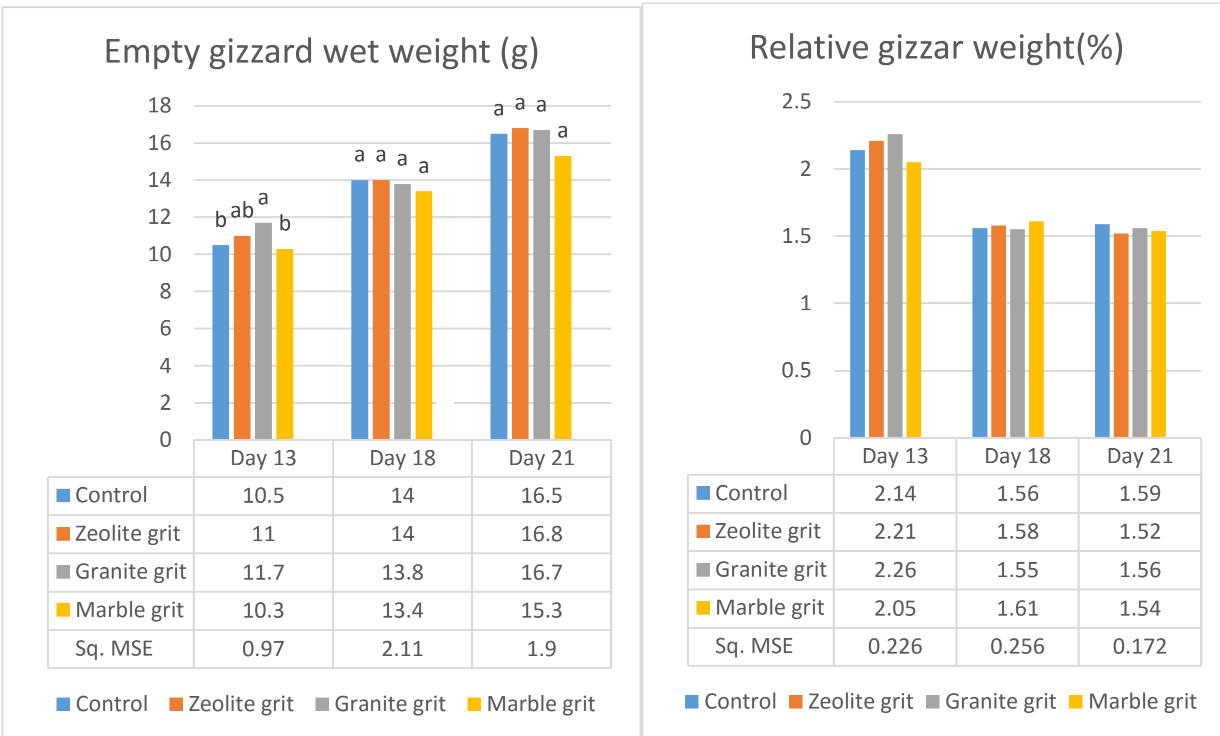
Gizzard relative content

Gizzard relative content from ZG was not significantly different from the relative content in MG at day 13, but the content in ZG was lower than CG and GG significantly.

Gizzard pH

Statistically, there was no significant difference observed in pH among all treatments at all dissection days. Comparing with gizzard pH value was 3.1 from CG on day 13, pH of gizzard from ZG was 3.5, which was the same as it from MG at the same day. Gizzard pH from ZG on day 21 was lowest than pH 3.0 in CG and 2.9 in GG.

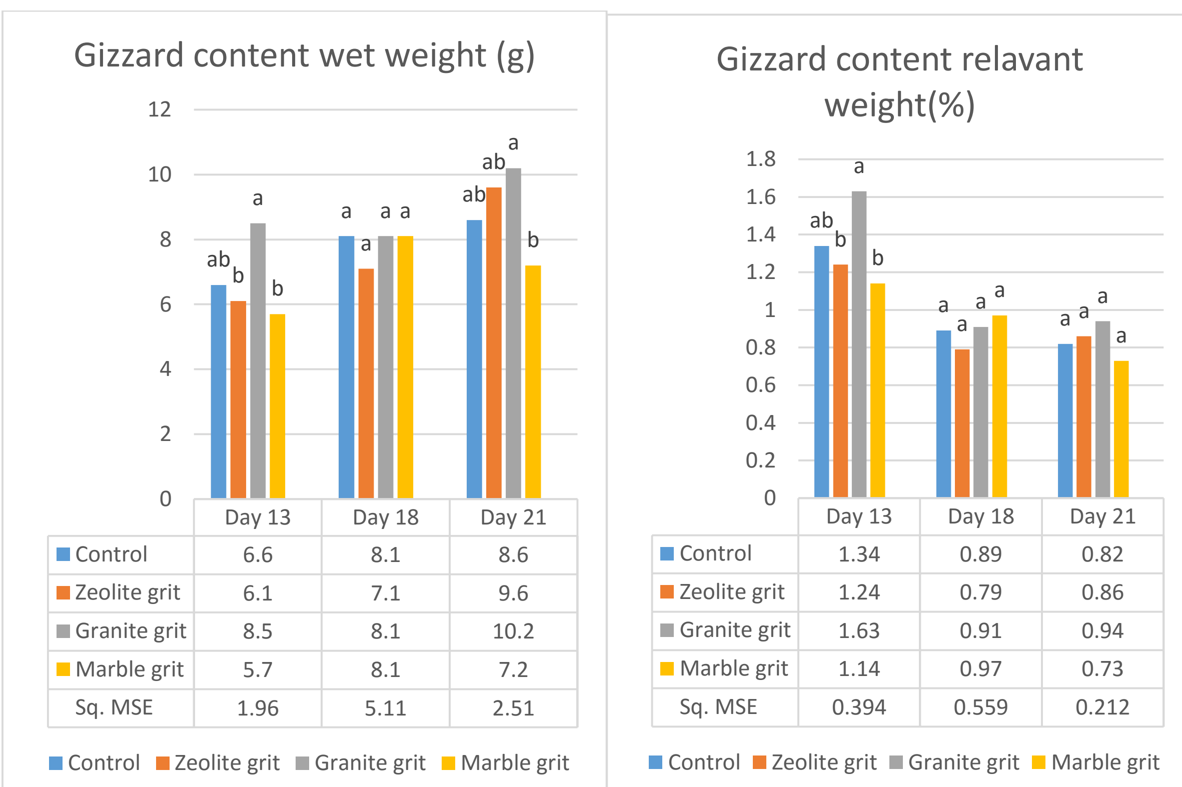
Fig 3: (**A**) Wet empty gizzard weight. (**B**) gizzard relative weight



	Day 13	Day 18	Day 21
Control	10.5	14	16.5
Zeolite grit	11	14	16.8
Granite grit	11.7	13.8	16.7
Marble grit	10.3	13.4	15.3
Sq. MSE	0.97	2.11	1.9

	Day 13	Day 18	Day 21
Control	2.14	1.56	1.59
Zeolite grit	2.21	1.58	1.52
Granite grit	2.26	1.55	1.56
Marble grit	2.05	1.61	1.54
Sq. MSE	0.226	0.256	0.172

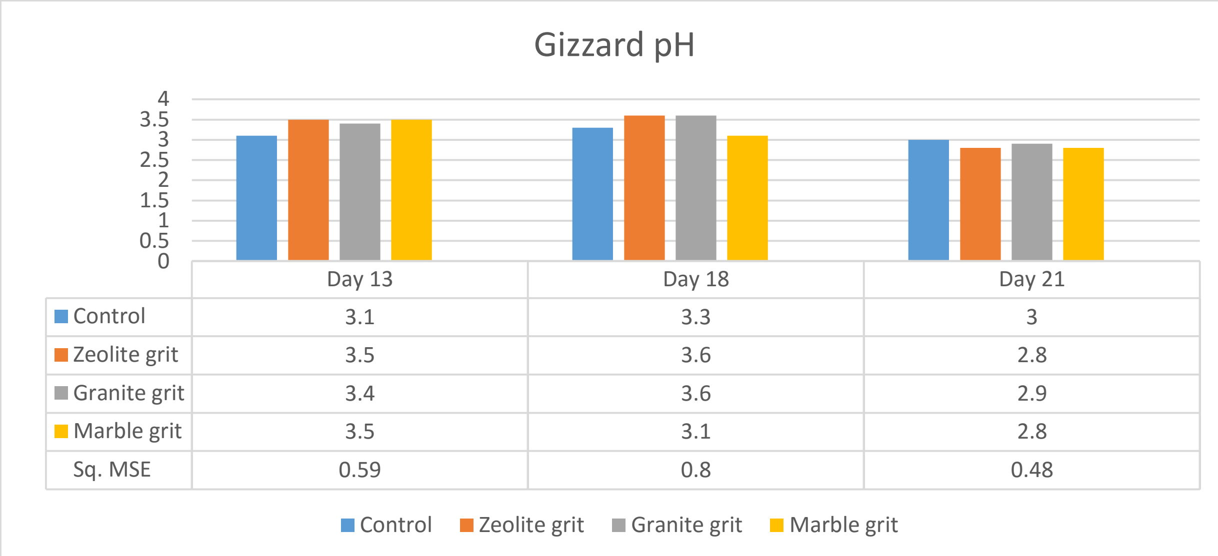
FIG. 4. Gizzard content wet weight and gizzard content relative weight (%)



	Day 13	Day 18	Day 21
Control	6.6	8.1	8.6
Zeolite grit	6.1	7.1	9.6
Granite grit	8.5	8.1	10.2
Marble grit	5.7	8.1	7.2
Sq. MSE	1.96	5.11	2.51

	Day 13	Day 18	Day 21
Control	1.34	0.89	0.82
Zeolite grit	1.24	0.79	0.86
Granite grit	1.63	0.91	0.94
Marble grit	1.14	0.97	0.73
Sq. MSE	0.394	0.559	0.212

Fig. 5. Gizzard pH on different dissection days



4.3 Excreta samples

Excreta samples were collected to measure AME and to get excreta particle distribution. The excreta particle size distribution was displayed by percentages from five size ranges; > 1.4 mm, 1.4-0.8 mm, 0.8-0.5 mm, 0.5-0.2 mm and <0.2 mm.

AME

AME value (**Table 3**) from day 13-18 and day 18-21 did not have significant difference among treatments.

Table 3: AME value from day 13-18 and day 18-21

AME value	Control Group	Zeolite Group	Granite Group	Marble Group	Sq. MSE	Significance
AME Day 13-18	13.5	13.6	13.7	13.6	0.4	0.776
AME Day 18-21	14	14.1	14.2	13.5	1.04	0.35

Excreta particle size distribution

All excreta particle size distribution (EPSD) was shown in percentage by each period separately.

Day 11-13

The percentage of different particle sizes from Day 11- 13 **(Fig. 6)** has significant difference among treatments. The percentage of size with larger than 1.4 mm from zeolite group has no significant difference from any treatment. The percentage of particle size between 1.4-0.8 mm from ZG was not different from the ratio of same size range from the control group.

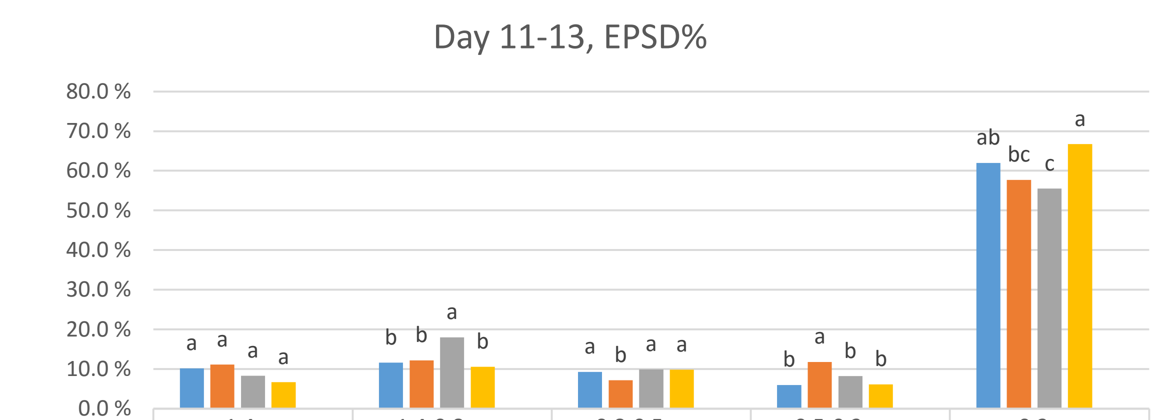
Day 13-18

The percentage of different particle size from Day 13-18 **(Fig. 7)** has no significant difference among treatments at same size range.

Day 18-21

At day 18-21 **(Fig. 8)**, excreta size with 0.8-1.4mm in ZG was significantly lower percentage than same size in GG but not significance than CG and MG. However, the percentage of smaller size (0.2-0.5mm) in ZG was higher than other three treatments significantly.

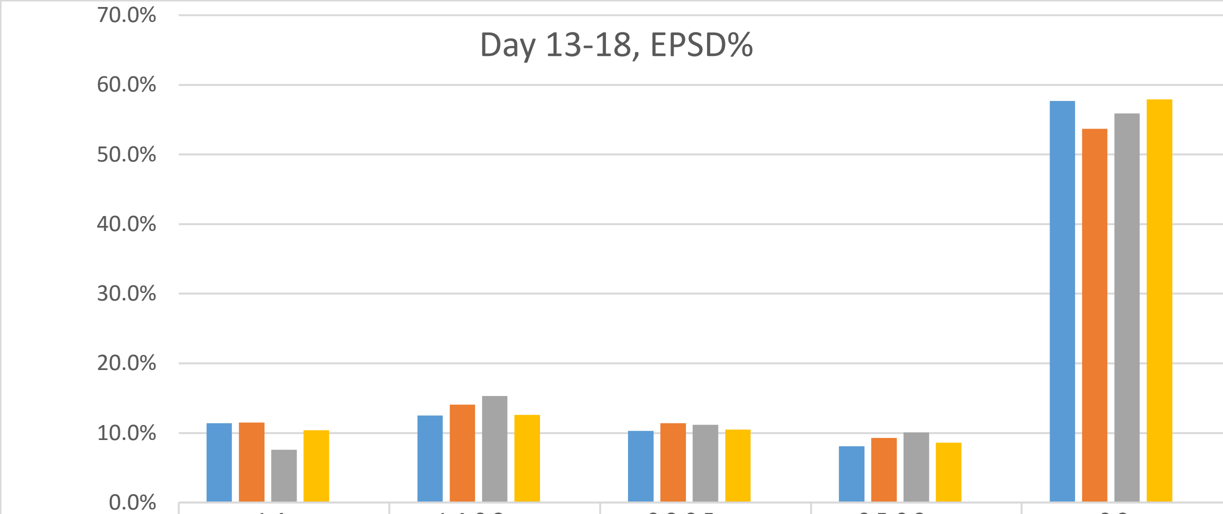
Fig. 6: Excreta particle size distribution from Day 11-13



	> 1.4 mm	1.4-0.8 mm	0.8-0.5 mm	0.5-0.2 mm	< 0.2 mm
Control	10.2 %	11.6 %	9.3 %	6.0 %	62.0 %
Granite grit	11.1 %	12.2 %	7.2 %	11.8 %	57.7 %
Zeolite grit	8.3 %	18.0 %	9.9 %	8.2 %	55.5 %
Marble grit	6.7 %	10.6 %	9.8 %	6.1 %	66.7 %
Sq. MSE	0.046	0.0202	0.0148	0.0274	0.0599

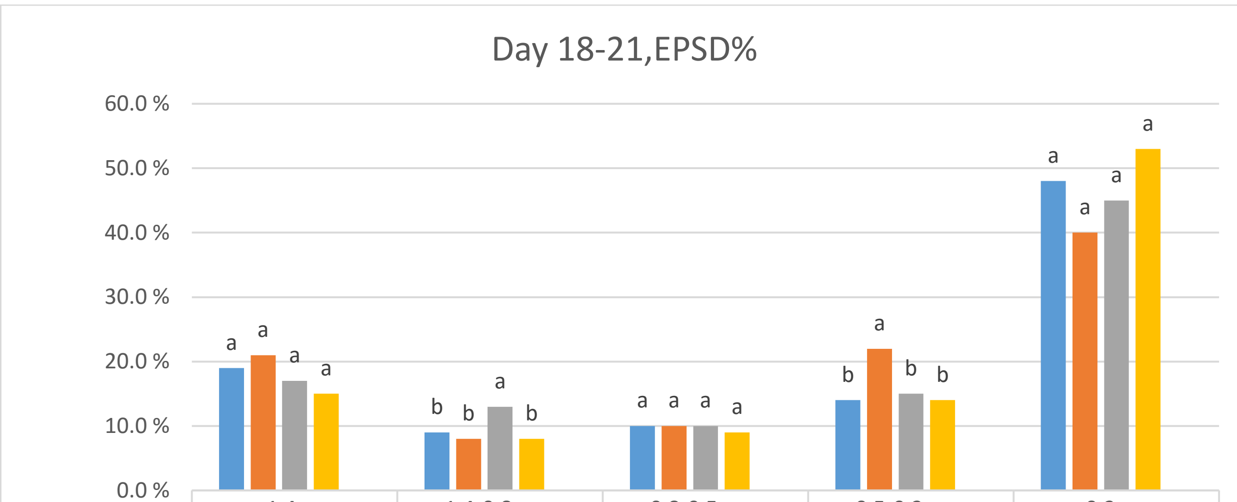
Control Granite grit Zeolite grit Marble grit

Fig. 7: Excreta particle size distribution from Day 13-18



	> 1.4 mm	1.4-0.8 mm	0.8-0.5 mm	0.5-0.2 mm	< 0.2 mm
Control	11.4%	12.5%	10.3%	8.1%	57.7%
Granite grit	11.5%	14.1%	11.4%	9.3%	53.7%
Zeolite grit	7.6%	15.3%	11.2%	10.1%	55.9%
Marble grit	10.4%	12.6%	10.5%	8.6%	57.9%
Sq. MSE	0.041	0.0323	0.0253	0.0323	0.1042

Fig. 8: Excreta particle size distribution from Day 18-21



	> 1.4 mm	1.4-0.8 mm	0,8-0.5 mm	0.5-0.2 mm	< 0.2 mm
Control	19.0 %	9.0 %	10.0 %	14.0 %	48.0 %
Granite grit	21.0 %	8.0 %	10.0 %	22.0 %	40.0 %
Zeolite grit	17.0 %	13.0 %	10.0 %	15.0 %	45.0 %
Marble grit	15.0 %	8.0 %	9.0 %	14.0 %	53.0 %
Sq. MSE	0.065	0.034	0.034	0.042	0.145

Control Granite grit Zeolite grit Marble grit Sq. MSE

4.3 Zeolite collection and its size distribution in excreta

Amount of ingested stones at day 5-11

From day 5- 11 **(Fig. 9)**, a total of 38 g/cage of each grit was supplied on top of the diet, but not all of stone were eaten. Birds in ZG and GG consumed 37 g and birds from MG only consumed 23 g.

Amount found in gizzard

The weight of zeolite in gizzards **(Fig. 10)**, was significantly heavier than marble on day 13 but significantly lower than granite on day 13 and day 18.

Grit passage rate and disappearance rate

On Day 5-11, grit passage **(Fig. 11)**, in ZG was similar amount of grit passage in GG but had significant higher than marble passage. On day 11-13, both grit passage in ZG and MG has significant higher than the passage in GG. At day 13-18, birds in ZG passed out grit significant lower amount of grit in GG. Grit disappearance in ZG was significant lower the disappearance of marble but significant higher the disappearance of granite.

Grit size distribution in excreta from different periods

Grit stone particle distribution (SPD) shows by periods. Comparing SPD in granite, zeolite and marble has lighter weight in bigger size rang and day 11-13. Later day 13-18, bigger size of zeolite has been reduced and at the same time the ratio in smaller size range was increased.

Comparing with weight ratio of size from 0.5-1.4 mm in ZG at day 13-18, they were not strong reduce the ratio in same size range.

Fig. 9: Amount of stone ingested by chicks

Fig. 10: Amount of grit in gizzard at different days

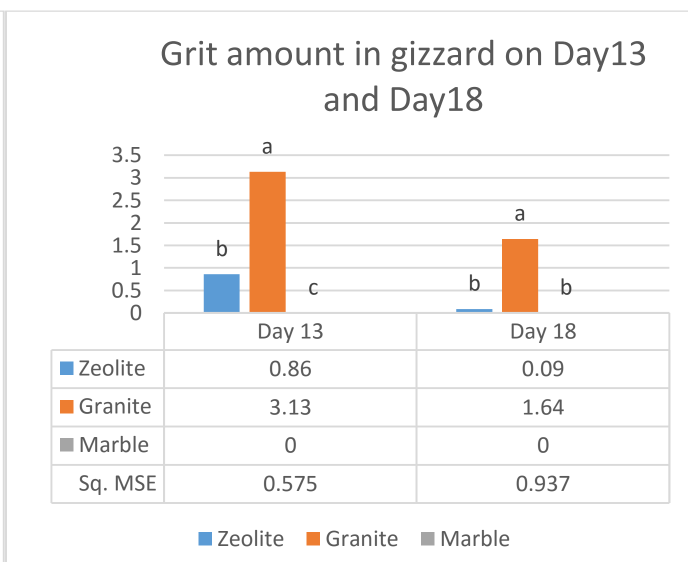
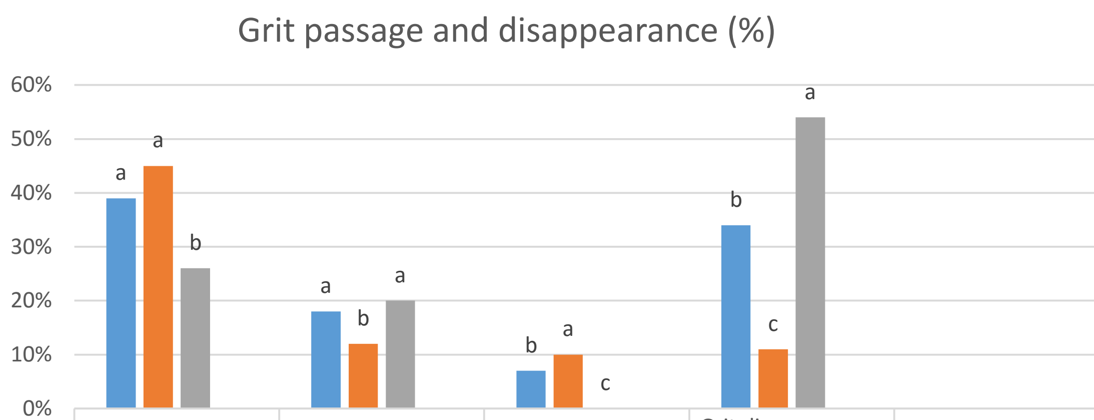


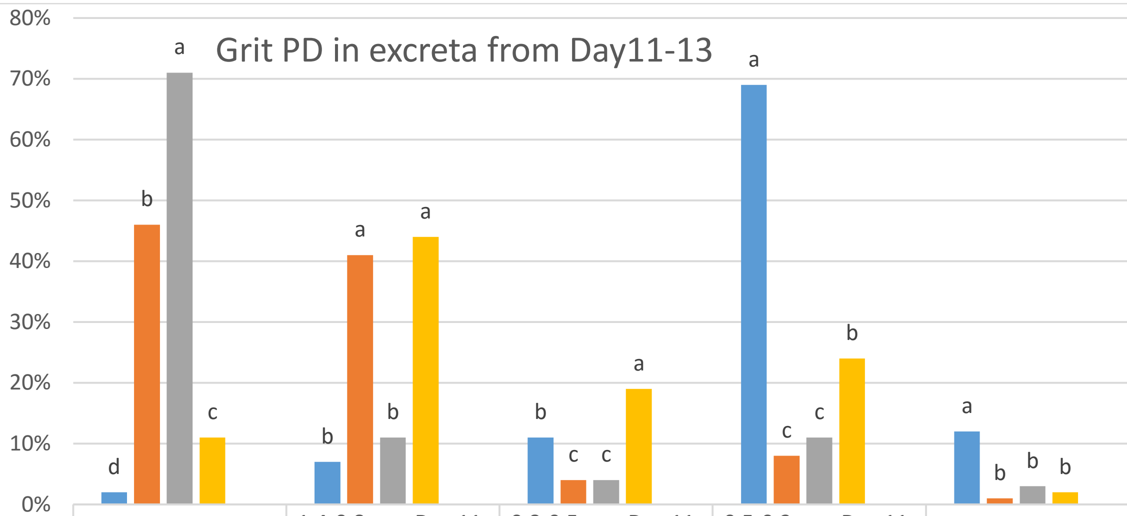
Fig. 11: Grit passage rate and grit disappearance rate



	Day 5-11	Day 11-13	Day 13-18	Grit disappearance 5-18	
Zeolite	39%	18%	7%	34%	
Granite	45%	12%	10%	11%	
Marble grit	26%	20%	0%	54%	
Sq. MSE	0.085	0.062	0.042	0.106	

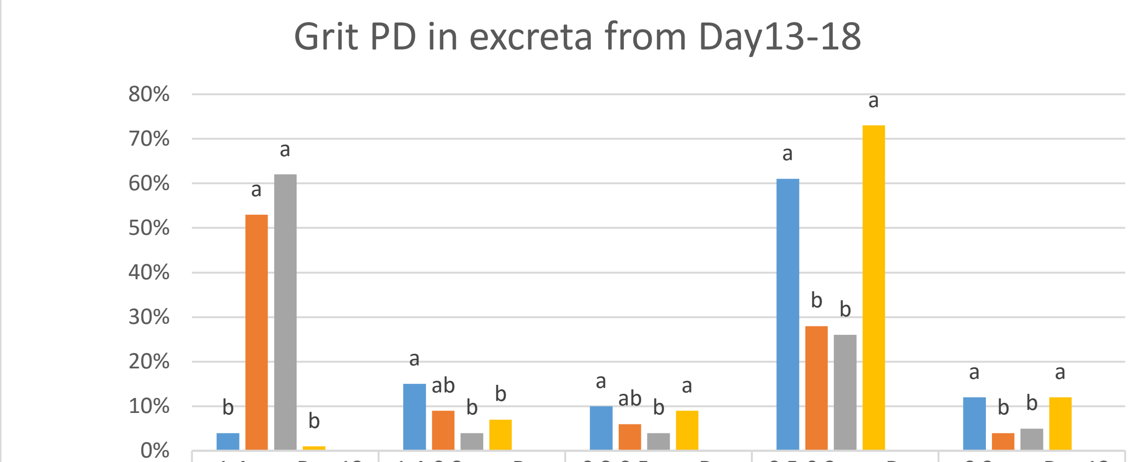
Zeolite Granite Marble grit

Fig. 12: Grit size distribution in excreta from day 11-13



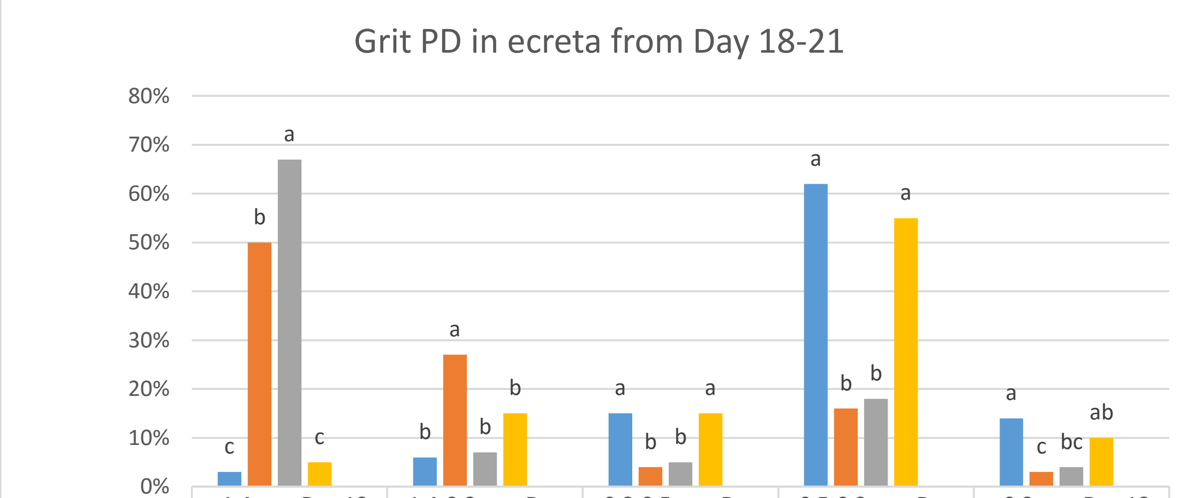
	> 1.4 mm Day 11-13	1.4-0.8 mm Day 11-13	0.8-0.5 mm Day 11-13	0.5-0.2 mm Day 11-13	< 0.2 mm Day 11-13
Control	2%	7%	11%	69%	12%
Zeolite grit	46%	41%	4%	8%	1%
Granite grit	71%	11%	4%	11%	3%
Marble grit	11%	44%	19%	24%	2%
Sq. MSE	0.09	0.07	0.06	0.09	0.07

Fig. 12: Grit size distribution in excreta from day 13-18



	> 1.4 mm Day 13-18	1.4-0.8 mm Day 13-18	0.8-0.5 mm Day 13-18	0.5-0.2 mm Day 13-18	< 0.2 mm Day 13-18
Control	4%	15%	10%	61%	12%
Zeolite grit	53%	9%	6%	28%	4%
Granite grit	62%	4%	4%	26%	5%
Marble grit	1%	7%	9%	73%	12%
Sq. MSE	0.14	0.08	0.04	0.14	0.05

Fig. 12: Grit size distribution in excreta from day 18-21



	> 1.4 mm Day 18-21	1.4-0.8 mm Day 18-21	0.8-0.5 mm Day 18-21	0.5-0.2 mm Day 18-21	< 0.2 mm Day 18-21
Control	3%	6%	15%	62%	14%
Zeolite grit	50%	27%	4%	16%	3%
Granite grit	67%	7%	5%	18%	4%
Marble grit	5%	15%	15%	55%	10%
Sq. MSE	0.09	0.09	0.07	0.13	0.06

5. Discussion

This study focuses on the effect of zeolite as grit stone and applied results about effects of granite since those two tests shared the same control group. Discussions of growth performance are shared at the end of this part for two reasons.

1. There was no significant body weight difference among all treatments at Day 21.

2. Growth performance results from synergism of a broiler interacted with diets and stones which I will discuss firstly.

5.1 Effect of zeolite on feed intake

Feed intake of birds in Zeolite Group (ZG), Granite Group (CG) and Control group (CG) was not significantly different. However, various feed intakes were reported, increased feed intake (Olver 1989), reduced feed intake(ÖZTÜRK, ERENER et al. 1998) and no effect on feed intake (Roland, Rabon et al. 1990). Our result indicates combination of three main reasons; filling effect (retention) of zeolite in the digestive tract, effect of feed characteristics and zeolites aiding gizzard grinding.

Firstly, our data showed that types of insoluble grit stone (zeolite) or stones inclusion have no significant filling effect when fed with commercial pellets because of having high grit passage. Birds consumed same mean weight grit in ZG and GG, but we did not observe that significant difference in feed intake between two types of stone treatments. This result contrasts with from HINNERS and ELLIOTT (1972) who mentioned that grit can take up space, and this may occupy space for feed in gizzards. However, higher grit passage rate from ZG (39%*37 g total consumption,) and GG (45%* 37 g total consumption) indicate that none of the insoluble stones did retain longer or reduce feed intake. This is also by the result from Itani (2015) who found grit stone inclusion in broiler diet did not show significance in feed intake among grit and non-grit treatments.

As Svihus (2011) described, pH in gizzards get lower because of structural components in gizzard could stimulate more secretion of digestive juice and also prolong retention time of feed particles before they are reduced down to certain size. So, our observation is not surprisingly where we have highest gizzard pH (3.5), and we have less gizzard content wet weight in ZG. It maybe because of higher mean pellets intake in ZG during day 11-13. Furthermore, higher feed intake in ZG did not significant increase gizzard content wet weight. This indicates less retention time of feed in gizzard caused by feed characteristics and relative softness of zeolite may have influenced feed passage, which I will explain further below.

Secondly, no variation in feed intake may have effected by the characteristic of pelleted feed. Reduced particles size of ingredients from a grinder (Engberg, Hedemann et al. 2002) and further reducing size of grinded particles in pellets press (Svihus, Kløvstad et al. 2004) could result in more fine particles which has short retention time in gizzard. Thus gizzard will join in feed regulation so that moistened and dissolved feed in upper tract were moved down to fill a gizzard (Svihus 2011). A similar result is also observed by having a higher trend of feed intake in ZG day 11-13, day 18-21 and reduced gizzard content wet weight at day 13 and day18.

Thirdly, relative hardness of Zeolite altogether with its insoluble characteristics also results in increased feed intake at day 11-13 and day 18-21, by being reduced size in the gizzard and shortening passage rate of feed on whole digestive tract. Comparing with data from GG, zeolite disappeared 34% ($P<0.0001$) and passed out 18% of 37g ($P=0.0065$) during day11-13. This significant disappearance rate in gizzard and faster passage in excreta explained that zeolite could push or drag feed further toward to a cloaca since feed and zeolite will effect volumetric flow in the same digestive chamber. Another reason having higher feed intake of ZG observed both at day 11-13 and day 18-21 could be explained by its insolubility, like fibre effecting passage rate (Roberfroid 1993). Insoluble fibre reduced overall feed retention time by having rapid passage after escaped from gizzard (Hetland, Choct et al. 2004).

Overall, less variation in feed intake among ZG, GG, and non-grit group result in the presence of mainly three reasons; not significant filling effect of soft zeolite, less retention time of insoluble zeolite and smaller particle size present in pellets. Those interactions among birds, diet and insoluble grit not only regulate feed intake but If so, more feed flow was needed to compensate space caused by interaction of zeolite consumption and its effect on gizzard performance.

Other species are not focus of this paper but it is worth to mention similar stories. Japanese quail (Savory 1980), will normally compensate for the lower nutrient concentration by increased feed intake. Laying hens, fed low nutrient density diets by diluting feed with NSP, sand and grit increased the feed intake. An experiment (Van Krimpen, Kwakkel et al. 2007) observed both hens' feed inclusion with fine sand and coarse grit did not affect feed intake, eating time or gizzard development.

5.2 Effect of zeolite on gizzard performance and excreta particle size distribution

In our experiment, no differ in broiler gizzard size were absorbed among ZG, GG and CG. It seems zeolite bring no changes in gizzard size as done by structural materials with elastic property like oat hulls, whole wheat and wood shaving (Hetland and Svihus 2001, Sacranie, Svihus et al. 2012). Also, it may be caused by less amount of stones are retained and stimulate because even granite (Van Krimpen, Kwakkel et al. 2007) did not stimulate bigger gizzard than zeolite did. Besides, it may result from a combination of previous reasons related to stones and characteristic of pellets diets which has many smaller particles with increasing passage rate. As I mention above about zeolite increased feed intake slightly higher, gizzard pH is acceptable since faster passage stimulates more feed intake, which make shorter retention time of digest in gizzard to hinder lowering pH. Gizzard holding capacity of feed is also not improved because faster passage of stone also increases emptying speed even when feed and water is ad *libitum*.

Gizzard in grinding may less effective than degradation of stone itself. Thus, we observed when ZG has more grit disappearance from day 11-13, the excreta smaller particles (0.2-0.5 mm) significantly increased in percentages than same size in from three other treatments; the excreta bigger particles (0.5-0.8 mm) has significantly lower in percentage than other treatments. The similar effect also observed from day 18-21 from our result. Worthily to note, we did not found a similar relationship during day 13-18 while no stone inclusion in this periods only. Also commonly known in Physic, when a solid item breaks, the ratio of surface area and volume will increase. This may also agree that stone characteristics (Rehakova, Čuvanová et al. 2004) are effecting the grinding efficiency. It is less gain to compare if conditions are various like layers or broilers but one study about grit and coarse ingredients in broiler can increase smaller digesta size in duodenum (Hetland, Svihus et al. 2003). If so, it is safety to say that relative softness of zeolite or similar structure has more benefits than harder stone on gizzard grinding.

5.3 Broilers' growth performance

At the end of the experiment, no significant weight gain among treatments. However, at the second half experiment, day 11-21, the weight gain in ZG was significant ($P<0.001$) higher than the gain in CG and MG. This result due to comprehensive interaction among, birds, feed and grit stones. This result from ZG is not a surprise because of total added value from higher feed intake from day 11-21, higher feed particles reduction (better digestion) from day 11-13 and 18-21, and higher zeolite disappearance by grinding.

It is worth to mention reports related to other type of grits. Smetana and Vale (1972) observed that no significant body weight difference among treatments when inclusion granite in broilers' commercial diets. Also, layers also has no significant difference in body weight, feed efficiency and egg production when supplement granite with both mash and mash-grain (50%: 50%) diets separately, according to a ten-months' test(Day, Dreesen et al. 1958). Also a report mentioned feed per gain in non-grit treatment was higher than granite, feldspar group but lower than calcium group(Day, Dreesen et al. 1958).

Logically, FCR is not a good expression to see if there a significant effect because the ratio of digestible protein and digestible protein. Even birds have higher utilization in macro nutrients, the excess part over maintenance energy will convert to fat, which has lower density than previous form, like proteins or starch in diets. Thus when higher digestibility in birds results in lighter weight gain, FCR cannot reflect details. Less valuable of FRC measurement also was suggested by Svihus (2011). However, in order to indicate zeolite's possible applications, relevant numbers are discussed. FCR in ZG at day 5-21 was 1.34, which is lower than other treatments 1.36 but the difference was not significant.

6. Conclusion and possible appilication

In clusion, zeolite as grit stone with a range of 1-2.5mm on cormercial pelleted diets have no negetive effect on birds weight gain, feed intake, feed untilization, gizzard size, gizzard pH and on feed size reduction. However, adding zeolite grit from day 5 results in significantly improve birds weight gain from day 11-21.

Limitation of our experiments are did not measure microorganisms in broiler since beneficial binding effect of zeolites can increase weight gain. Also, we did not measure fat content of chicken, it is hardly say that digestibility may has significant difference but when birds absorbed energy in diets and converted into fat in body it may reduce the weight (gain) differences by changing feeds to low density fat.

Those two and results from our data may indicate:

Providing zeolite grit to broilers from day 1 may start to see significant weight gain on earlier stage.

Providing zeolite 0.95g/bird/day (mean value of grit consumption from day 5-11) or higher dose may cause feed waste in poultry industry since no significant benificial effect on end products.

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