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Effect of feeding diet with whole wheat with and without grit stones on performance and digestive characteristic in broiler chickens

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Abstract

An experiment was conducted to study the effect of feeding the diet with whole wheat with and without grit on broiler performance and digestive characteristics. The trial consisted of four treatments: one control and three grit treatments. 250 1-day-old broilers were randomly placed in four pens. Commercial starter diets were fed from 1 to 10 days of age, commercial grower diets from 11 to 18 days of age and starter diet containing 15 % of whole wheat from 18 to 21 days of age. From five days of age, four birds were randomly placed in each of 48 cages. Birds in 12 cages were given no grit, zeolite grit, granite grit or marble. The grit was given on top of the feed at 5, 7 and 9 days of age, and each cage was offered 38 g each type of grit in total. On day 18, 19 and 20, one gram of each type of grit has given per bird in each treatment. At 5, 11, 13, 18 and 21 days of age, feed intake and body weight were registered. At 13, 18 and 21 days of age, one randomly selected bird from each cage was killed and dissected. The gizzard and crop content were collected and frozen for later analysis. Empty gizzard weight and full gizzard weight were weighed. The small intestine was also collected and frozen. At 11, 13, 18 and 21 days of age, the excreta was collected for analysis of particle size distribution of grit and excreta. On day 21, six birds from each treatment were given 50 g whole wheat after fasting 10 hours. After giving feed for 2 hours, small amount of excreta was collected and the whole wheat was measured. The amounts of whole wheat in different digestive segments that form 21 days of age were measured. The findings showed grit supplementation did not impair nor improve broiler overall performance compared to control treatment. Gizzard weight and gizzard content weight were not increased due to providing grit except heavier gizzard was observed at 13 days of age in granite group compared to control group. Gizzard pH was not affected by grit addition. The proportion of larger excreta particle size (>1.4mm) and smaller particle size (<0.2) fraction were not influenced by grit. No significant difference in the proportion of whole wheat grinded in the gizzard between the grit group and the control group.

Keywords: Whole Wheat, Grit stone, Gizzard, Particle Size.

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Table of Contents

1. Introduction.....	1
2.literature review	2
3. Material and Methods	6
3.1 Animal housing and dissection	6
3.2 Feed and grit stones	8
3.3 Laboratory working	11
3.3.1Excreta	11
3.3.2 AME.....	13
3.3.3 Gizzard content	13
3.3.4 Measurement of Whole-wheat kernels	14
3.3.5 Calculation	15
3.4 Statistical analysis.....	16
4. Result	16
4.1 Broiler performance parameters	16
4.2 Dissection data	17
4.3 Excreta particle size distribution.....	20
4.4 Whole wheat in different digestive segments	21
5. Discussion	21
5.1 Effect of grit on grinding and excreta particle size distribution	21
5.2 Effect of grit on performance of broilers	22
5.3 Effect of grit on gizzard development	24
5.4 Effect of grit on AME	26
6. Conclusion	27
7. References.....	28

1. Introduction

Broiler chickens are commonly fed finally ground pellets feed. In this case, grains for the poultry diet need to be dried and ground, which increases in feed production cost. However, coarser grinding or the using whole cereals in the diet reduce feed milling cost. Furthermore it has been revealed that this feeding system, in some case, has some positive effect on bird production (Hetland et al. 2002; Plavnik et al. 2002; Svihus et al. 1997a). The beneficial effect of coarse ingredients has been linked to a more developed gizzard that ensures a complete grinding and a well-regulated feed flow and secretion of digestive juices (Svihus et al. 2010).

Birds have a behavior that instinctively consumes small grit from the environment and there has a long history of interest in grit used by avian gizzard. It has been believed that birds consuming smaller grit generally use to improve mechanical grinding of food in the gizzard (Balloun & Phillips 1956; Hetland et al. 2002; Moore 1999). Therefore it is possible that providing grit to birds when the diet including whole cereals will improve grinding action of the gizzard and thus improving feed utilization.

The main objective of this study is to test the hypothesis that grit acts as grinding agent to facilitate gizzard function in whole grain diets of domesticated poultry. This is done by comparing the excreta particle size distribution between birds fed a whole-wheat diet with and without grit. Furthermore, the effect of the grit on the development of the gizzard and performance of broilers also investigated when the diet including whole wheat.

2.literature review

Poultry can be defined as domestic fowls, including chickens, turkeys, geese and ducks, raised for the production of meat and egg. Worldwide, more chickens are kept than any other type of poultry. The utilization of feed in chickens is affected by several factors, such as age, sex and genetic differences, feed and feed structure, which is considered to influence the performance of birds. Digestive tract is a main component for animals to accomplish the essential process of food intake, including storage, digestion, nutrients absorption and waste elimination. In order to make a highly specialized broiler diet it is important to understand their digestive tract structure and function.

When compared to other domestic animals, chicken's digestive tract has several unique characteristics, such as a crop, gizzard, caeca and cloaca. A Crop is the main storage compartment of birds, which plays an important role for moistening and softening feed, but little digestion takes place here (Jackson & Duke 1995). The first significant amounts of digestive juices, pepsinogen and hydrochloric acid, are secreted in the proventriculus, which is an enlargement of the esophagus. The proventriculus has limited mobility and the feed particles combined with digestive juices pass through it quickly to enter the gizzard. The gizzard is a highly muscular organ, which is composed by two thick and two thin muscles.

The primary function of the gizzard is to replace the mastication role of teeth to breaking down large feed particles to increase the surface area of the feed and thus allowing the feed more accessible for the digestive enzymes (Gionfriddo & Best 1999; Svihus 2011). Hetland et al. (2002) reported that digesta passing through the gizzard

have a remarkably consistent particle size distribution, regardless of the original feed structure. It indicates that the food particles have to be ground to a certain size before they leave the gizzard into the small intestine through the opening of the pyloric sphincter (Duke 1992; Hetland et al. 2003; Moore 1999; Svihus 2011). In addition, the gizzard regulates feed flow and chemical degradation of the feed (Svihus 2011). Since the very small volume of proventriculus resulting in a short retention time, the majority of the chemical digestive action therefor takes place in the gizzard. The digestive particles pass into the small intestine when ground sufficiently and a reflux of intestinal digesta into the gizzard can also occur. When the gizzard muscle contracts, it also pushed gastric materials into proventriculus, which allows for additional digestive juices to be added into the contents (Duke 1992). The mean retention time of the feed in the proventriculus and gizzard has been estimated between half an hour and an hour(Svihus 2011). However, larger particles and high fiber components are selectively retained for a longer time in the gizzard(Hetland et al. 2005; Hetland et al. 2003). Although the retention time of non-structure component does not influence by the structure component in the diet, the mean retention of the feed will increase when the diet contains larger particle components (Svihus 2011). The diet retention time in the anterior digestive tract is vital for controlling the ratio at which these get in touch with digestive enzymes and absorptive surface. Furthermore, the gizzard has been assumed to have an important role as a feed intake regulator. As Svihus (2011) described, a well-functioned gizzard may hinder feed overconsumption simply due to the physical constraints in gizzard volume combined with limitations to feed passage from the gizzard to the duodenum.

The gizzard is known to respond rapidly to changes in the diet, and this is a consequence of changes to the structure of the diet (Moss 1989; Moss & Trenholm 1987; Svihus 2011). The gizzard needs to work harder when the birds are feeding on coarse food that consequently leads to increase in the gizzard size. It has been reported that including structural component in the diet, such as coarse grounding or whole cereals, oat hulls and wood shaving increased in the gizzard size and increased

in nutrient utilization (Hetland et al. 2003; Preston et al. 2000). The volume of the gizzard also increases substantially when structure components are included in the diet (Amerah et al. 2009; Amerah et al. 2008).

The structure of feed has a strong influence on the performance of broiler chickens. It is generally accepted that feeding pelleted diets improve broilers growth rate with an increased feed intake (Nir et al. 1994). It also has been observed an improved feed conversion with pellet feeding (Engberg et al. 2002). However, high intake may result in lower starch digestibility due to overload of wheat starch in digestive tract (Svihus & Hetland 2001). Furthermore Engberg et al. (2002) has been reported that significantly highest mortality was found in birds fed the finely ground pelleted diets than mash or coarsely ground diet. The author also indicated that feeding grinding and pelleting diet reduced in relative gizzard weight, which is mainly due to the lack of mechanical stimulation by the feed.

In recent years, whole grains including in the diet has received interest in both the scientific community and the commercial practice. The use of whole grain is not new, and early research indicated that there is no consistent improvement in the feeding value for broiler chickens when wheat, barley, oat or maize is ground (McIntosh et al., 1996). For wheat, Rose et al. (1995) reported that broiler chickens fed whole wheat diets has the same growth rate and feed conversion as birds feed pelleted wheat diets.

Wheat is a major ingredient in chicken diet due to its high protein and starch content. The major benefit of utilizing whole cereals is reducing the feed cost due to reduced grinding and processing, which in turn lowers the cost of producing broilers. Furthermore, studies have been shown that whole cereals in the diet have a beneficial effect on gizzard development and feed utilization (Preston et al. 2000; Svihus et al. 1997b; Svihus & Hetland 2001). An enlarged gizzard has been observed when whole wheat included in the diet (Biggs & Parsons 2009; Hetland et al. 2002; Preston et al. 2000; Svihus & Hetland 2001). The increase in size of the gizzard is reasonable when

feeding whole-wheat due to more extensive grinding of food within the gizzard and thus increased size of the two pairs of gizzard muscles.

When the diet included whole cereals the extra grinding process of the gizzard requires energy(Hetland et al. 2002). Consequently, less energy would be expected to use for growth with whole cereal diets than pellet diet. However, including whole wheat in the diet did not influence feed conversion efficiency (FEC) (Hetland et al. 2002; Preston et al. 2000). Furthermore in some studies diet contains whole wheat has been observed increased in FCE (Plavnik et al. 2002). Svihus and Hetland (2001) and Hetland et al. (2002) found that starch digestibility was increased when whole wheat included in the diet, which followed by reduction of feed intake. Svihus et al. (2000) mentioned that starch digestibility is negatively correlated with the wheat consumption and the author (Svihus 2011) suggested that feeding whole wheat could eliminate an overload of starch due to its regulatory effect on feed flow through the limited capacity of gizzard for grinding seeds. It indicates that an active gizzard may be the important organ for preventing starch overload and plays an important role increasing starch digestibility. These authors conclude that a well-developed gizzard due to whole-wheat stimulation may response for a higher utilization rate of energy and nutrients.

In fact, the diet for wild ancestors of our domestic poultry consumes a lot of whole seeds and fibrous materials. Many of these birds ingest small stone from the environment to aid grinding in the gizzard (Gionfriddo & Best 1999). The grit found in avian gizzard thought to contribute to breaking down the feed. While sometimes is also acting as a mineral source (Gionfriddo & Best 1999). Early studies showed a clear beneficial effect of grit stone on growth rate, feed conversion efficiency and egg production of chickens, particularly when whole grains included in the diet(Balloun & Phillips 1956; Scott & Heuser 1957). On the other hand, several studies conducted more recently also failed to prove the beneficial effect of girt on performance of both layers and broilers. (Hetland et al. 2003; Itani 2015; Svihus et al. 1997a).

In practice, there is two types of grit are frequently used as dietary supplements: insoluble and soluble grit. Insoluble grit such as granite or silica remains completely undigested in the digestive tract of the birds. Studies in poultry have been shown that insoluble grit remains in the gizzard to assist in the mechanical grinding to reduce food particles to facilitate the digestive process(Gionfriddo & Best 1999; Hetland et al. 2002; Moore 1999). In contrast, soluble grit such as cuttlebone, limestone is usually completely digested by birds. For this reason, it has less capacity to enhance gizzard function. However, it has been thought to be a good source of supplementary minerals such as calcium(Taylor 1996). In addition, grit in the gizzard may also enhance digestion through further stimulating the secretion of digestive fluids or by facilitating the action of these fluids(Mcintosh et al. 1962).

3. Material and Methods

An experimental was conducted in the Animal Production Experimental Centre, at the Norwegian University of Life Sciences (NMBU), Ås, Norway, from 12th of the November to 4th of the December 2015. The experiment was a comprehensive study were performed in collaboration with five other master students, Kari Borg, Biemujiafu Fuerjiafu, Cecilia Larsson, Huan Liu, and Sodbilig Wuryanghai and not all the data from this experiment was presented in the current study. The main objective of the current paper is to detect an interaction between whole wheat and grit stone. The data from marble was excluded in this article.

3.1 Animal housing and dissection

3.1.1 Feeding period without whole wheat

250-day-old broiler chickens (ross 308) were placed into four equal sized pens (72cm×145cm) until 5 days of age. The floor of the pens was covered with a thick layer of wood shaving. Birds fed commercial starter diet and water ad libitum during

this period with a 24 h light. Extra heat provided using lamps over the pen to maintain the room temperature at approximately 28°C during the first week and reduced to 22°C over the three following weeks.

From five days of age, four birds were placed randomly in each of 48 quail cages (d.35cm×w.50cm×h.20) with a mesh floor. (Birds below 130 g were excluded from the experiment and these remaining birds were raised in the pen and did not participate further in the entire experiment.) Each cage had two feeders, one is for water and the other is for water and a tray located under the cage to collect excreta. Feed and water were filled 2 times per day to make sure chickens were always had enough food and water. Birds in each 12 cages given either no grit, grit made from granite, grit made from a softer type of rock (zeolite) and grit made from limestone (marble) along with the commercial pelleted diet. From 5 to 11 days of age birds given starter diet and switched to grower diet from 11 to 18 days of age. The grit stones were given on top of the feed. 8 g of each type of grit given to each of 12 cage at 5 days of age and 15 g of each type of grit given to each of 12 cage both at 7 and 9 days of age (birds in per cage were given 37 g grit in total during 5 to 11 days of age).

Birds weight were registered at 5, 11, 13 days of age. Simultaneously, feed consumption was measured. All Excreta on the trays from 5 to 11 and 11 to 13 days was collected in the morning at 11 and 13 days, respectively, and froze them immediately. These contents were used to determine the amount of grit in the excreta plus the particle size of excreta. Feed residues from 11 days were sieved to determine the consumption of grit during 5 to 11 days. The excreta was also collected at day 15, 16, 17 and 18 and pooled together.

At 13 and 18 days of age, one bird from each cage (in total 48 birds each day) was killed randomly by a cranial blow followed by a cervical dislocation and dissected. The digestive tract, including ceca, was removed from the bird, and put into a plastic bag and immediately frozen. The gizzard was weighed full and empty and the gizzard

content was put into a container and frozen.

3.1.2 Feeding period with whole wheat

The remaining birds (2 birds in each cage) from the feeding period that without whole wheat were given starter diet mixed with 15% whole wheat and per bird given 1 g each type of grit on day 18,19 and 20. The excreta were collected on day 20. On day 20, feed was taken away at 21:00 and the birds were starved until the next day at 7:00. The feed residues were collected and the remaining grit was measured. On day 21, one bird from each cage was removed from each quail cage, marked with its cage number, and birds from the same treatment were placed into same pen (4 per in total). Before giving access to feed, the trays were removed and excreta was collected, which is pooled together with the faeces from day 20. Excreta were used for excreta particle size distribution during 18 to 21days of age. 6 birds from each treatment remained in cages (24birds in total) were given 50 g whole wheat whereas another 6 birds from each treatment (24 birds in total) were given 50 g grower diet for 5 hours. After giving feed for 2 hours, the clean trays put back under the cage and the excreta produced during following 3 hours were collected into the small container. After giving feed 5 hours birds in the cage from each treatment (48 in total) was killed by a cranial blow followed by a cervical dislocation and dissected. The feed was taken out when birds were killed and weighed the feed residues. The crop and the gizzard contents put into a container, and the gizzard was weighted full and empty.

After dissecting all birds the remaining 12 birds located in the pens were put back into cages for further treatment. (since the further treatment not related to this paper the following experiment plan is not presented here.)

3.2 Feed and grit stones

Commercial diets were bought from the Norwegian feed company Norgesfôr and the

whole wheat was supplied from Felleskjøpet.

3.2.1 Quartz

The quartz 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 1.

Table 1: Average values for the chemical composition of quartz 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 grit with the dimension between 1mm to 2.5mm was ordered from ZEOCEM. The chemical composition of the zeolite was provided in table 2 by EL spol. Sr.o. Division of laboratory Service on 11.01.2016. The lab result analyzed 34 types of different chemical composition and only main elements are shared here.

Table 2: Average values for chemical composition of zeolite grit (ZEOCEM 2016).

SiO ₂	Silicon dioxide	68.54%
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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 with dimension between 0.5-2 mm was provided from Visnes Kalk AS in Lyngstad of Norway. The chemical composition of the marble (calcium carbonate CaCO₃) is shown in table 2.

Table 2: Average values for the chemical composition of marble (Visnes Kalk AS 2007).

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

3.3 Laboratory working

3.3.1 Excreta

Excreta samples were used to measure excreta particle size distribution, the amount of grit stone in the excreta and AME calculation. Before doing all these measurements, all faces samples were homogenized and representatively samples were taken out dried in an oven ($105\pm 2^{\circ}\text{C}$) over night to determine the dry matter content of excreta (this dry matter was used to calculate the weight of faeces used to wet sieving as dry matter base).

3.3.1.1 Excreta particle size distribution

Faeces from 11-13, 13-18 and 18 to 21 days of age were used to determine Excreta particle size distribution by wet sieving. 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 with 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.

Approximately 100 grams of homogenized excreta sample was dissolved in the water for 10 minutes with the assistance of a magnetic stirrer (IKA C MAG HS7) in a plastic box, and then samples with water poured into sieve stacks with the sieve size order 1.4, 0.8, 0.5 and 0.2 mm from top to bottom. Sieve stakes were shaken by Retsch sieve shaker (AS 200 Control) with amplitude 1.50 mm/g. The sieves were shaken 2 min with water at maximum pressure and 1 min without water to shake off excess water. Each sieve was then weighed with content. Before samples were sieved, sieve stacks were sieved as same procedure and weight of each empty sieve was registered in order to calculate net weight of faeces on each sieve (equitation 1). From 4 replicas per treatment, a subsample (approximately 2.5 grams) was taken out from

each sieve to determine dry matter (The average dry matter dry matter was used to calculate the respective weight of faeces in each sieve as dry matter base). To estimate a “wet tare sieve weight”, empty sieves were shaken as same procedure as faeces samples. The average of 11 registrations was used when subtracting the tare weight 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.

3.3.1.2 Grit in the excreta

The faeces content collected from each sieve was used to determine the amount of grit in the excreta. The separation of grit in faeces from each sample was using the floating method. This 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 (Faeces particles) float up and were washed out, while the high-density particles (Grit stone) are left in the bottom of the bowl. The grit stones were then collected in a container and dried at room temperature overnight and weighed.

Each type of grit stone found in excreta from each period was dry sieved to determine the grit particle distribution (net weight of grit on each sieve), which is used to calculate excreta particle size distribution without grit (proportion of excreta in each sieve accounts for total faeces). Each type of grit stones was sieved 4 replicates to get an average particle distribution.

Dry sieving procedure:

The same size sieves were used as wet sieving, which is 1.4, 0.8, 0.5 and 0.2 mm. The tare of each empty sieve was first registered before each sample was sieved. Each grit stone sample was dry sieved on the Retsch sieve shaker (AS 200 Control) for 1 minute with amplitude 1.00 mm/g. Each sieve was then weighed and registered with

content and the sieves were emptied again. And then the grit weight distribution was calculated (Equation 1). And then the proportion of different particle size of excreta accounts for the total amount of faeces was calculated (Equation 2).

Equitation 1

$$\text{net weight of grit in each sieve } g = \text{weight of each sieve with content } g - \text{weight of each empty sieve } g$$

Equitation 2

$$\begin{aligned} & \text{Excreta particle size distribution without grit (\%)} \\ & = \frac{\text{net weight of excreta in each sieve } g(DM) - \text{net weight of grit in each sieve } g}{\text{net weight of excreta used for wet sieving } g(DM) - \text{total amount of gritound in excreta}} \\ & \quad \times 100\% \end{aligned}$$

3.3.2 AME

Faeces from 13 to 18 days and 18 to 21 days were used to calculate apparent metabolizable energy content of the feed (equation 3). A certain amount of representative homogenized faeces and feed samples from each corresponding period were dried overnight ($105 \pm 2^\circ\text{C}$) and put them into bomb calorimeter (PARR 6400 Bomb Calorimeter) to measure the energy content of each sample.

Equitation 3

$$\begin{aligned} & \text{AME MJ/kg} \\ & = (\text{energy content in the feed MJ/kg} \times \text{total amount of feed consumed kg} \\ & \quad - \text{energy content in the faeces MJ/kg} \times \text{total amount of excreta}) \\ & \quad / \text{total amount of feed consumed kg} \end{aligned}$$

3.3.3 Gizzard content

The gizzard content from day 13, 18 and 21 was used to determine the amount of stones in the gizzard and gizzard pH. Gizzard pH was measured by VMR pH measurement. Before determining the amount of grit in the gizzard, gizzard content had been dried overnight in the oven ($150^{\circ}\text{C}\pm 2^{\circ}\text{C}$) to measure the dry matter. Due to a relatively small amount gizzard content, the whole sample had to be used for dry matter determination. Thus, the dried gizzard content had to be dissolved in water to facilitating floating method. The floating methods were performed the same procedure used in stone separation from faeces. The grit stone collected from gizzard dried at room temperature overnight and then the weight was registered.

In addition, the gizzard content from the birds given 50 g whole wheat at day 21 was also used to determine the amount of whole-wheat kernels by picking up wheat kernels manually into small containers from the dissolved gizzard contents. This step had been done before washed out the gizzard girt stone.

3.3.4 Measurement of Whole-wheat kernels

Crop content, small intestine and excreta that collected from the birds given 50 g whole wheat at day 21 were used to determine the amount of whole-wheat kernels. The crop content was dried in an oven ($150^{\circ}\text{C}\pm 2^{\circ}\text{C}$) overnight and weighed to get dry matter weight of whole wheat in the crop. The small intestine (three section) was thawed at room temperature and was placed on the board to open it. After opening throughout its length with a scissor, whole-wheat kernels were picked up by manually into small containers with tweezers.

Excreta was dried in an oven ($150^{\circ}\text{C}\pm 2^{\circ}\text{C}$) overnight and weighed and then dissolved in water until easy to pick up whole-wheat kernels and put them into small containers. All containers that used to fill whole-wheat kernels are marked with corresponding cages and treatments. And these containers with whole-wheat (from gizzard, intestine, excreta) dried in an oven ($150^{\circ}\text{C}\pm 2^{\circ}\text{C}$) overnight followed by measuring the net dry

matter weight of whole-wheat kernels.

3.3.5 Calculation

Calculation related to whole wheat

1. *Whole wheat passed crop DM g*

$$= \text{whole wheat consumption DM g} - \text{whole wheat in the gizzard DM g}$$

2. *Whole wheat passed gizzard DM g*

$$= \text{whole wheat consumption DMg} - \text{whole wheat in the crop DM g} \\ - \text{whole wheat in the gizzard DM g}$$

3. *amount of whole wheat grinded down DMg*

$$= \text{whole wheat consumption DMg} - \text{whole wheat in the (crop} \\ + \text{gizzard} + \text{small intestine} + \text{excreta) DMg}$$

4. *amount of wholewheat grinded*

$$\text{expressed as percentage of whole wheat passed crop \%} = \\ \text{whole wheat grinded DMg} / \text{whole wheat passed crop DMg} \times 100\%$$

Calculation related to grit

1. *grit passage during (n) period %*

$$= \frac{\text{grit found in excreta form each cage during (n) period g}}{\text{total amount of grit consumed by birds in each cage during 5 – 11 day g}} \\ \times 100\%$$

(n=5 to 11 days of age; 11 to 13 days of age; 13-18 days of age.)

2. *grit grinded %* = $(a - b - c - d) / a \times 100\%$

a: total grit consumed by birds in each cage during 5-11 day (g)

b total amount of grit found in excreta in each cage during 5-11 (g)

c: grit in gizzard found at 13 day (g)

d: grit found in gizzard at 18 day (g)×3

3.4 Statistical analysis

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

4. Result

4.1 Broiler performance parameters

Broiler performance parameters including body weight gain, feed intake and feed per gain ratio for different treatments (control, zeolite and granite) during different periods (5 to 11, 11 to 13, 13 to 18 and 18 to 21 days of age) are presented in table 1. The broiler cumulative performance data for the overall experimental period (5 to 21) and the AME content (13 to 18 and 18 to 21 days of age) are also included in Table 1.

During 5to11 days of age, No significant difference ($P>0.05$) observed between body weight gain, feed intake and feed-gain ratio between all treatments. No significant difference ($p>0.05$) between treatments was observed in body weight gain, feed intake and feed-gain ratio both during 11 to 13 and 13 to 18 days of age, Zeolite-fed birds had higher feed intake compared to control and granite-fed group from 13 to 18 days. However the difference was not significant ($P=0.05$).

No significant difference ($P>0.05$) between the control group and grit group was observed in body weight gain, feed intake and feed-gain ratio during 15% whole wheat feeding period (18-21days of age).

During overall 5-21 days experiment period, given grit to birds did not seem to improve nor impede growth rate and feed utilization ($p>0.05$).

No significant difference ($p>0.05$) was noticeable in the AME content between all treatments both in the period from 13 to 18 and 18 to 21 days.

Table 1. Weight gain, feed intake and feed/gain from different period

5-11 days (starter diet)	Control	Zeolite	Granite	Sq.MSE ²	P-value
Weight gain g	225	219	225	15.8	0.5579
Feed intake g	275	269	271	10.2	0.3487
Feed/gain	1.23	1.23	1.21	0.053	0.4569
11-13 days (grower diet)	Control	Zeolite	Granite	MSE	P-value
Weight gain g	139	145	145	10.7	0.2276
Feed intake g	161	170	165	9.9	0.054
Feed/gain	1.16	1.18	1.14	0.057	0.3162
13-18 days (grower diet)	Control	Zeolite	Granite	MSE	P-value
Weight gain g	384	406	384	24.7	0.0758
Feed intake g	495	516	496	26.9	0.1376
Feed/gain	1.29	1.27	1.29	0.038	0.3680
AME MJ/Kg	13.5	13.6	13.7	0.40	0.776
18-21 days (15% ww) ¹	Control	Zeolite	Granite	MSE	P-value
Weight gain g	135	152	143	28.2	0.1182
Feed intake g	270	281	283	29.6	0.0938
Feed/gain	2.05	1.88	2.07	0.409	0.8305
AME MJ/Kg	14.0	14.1	14.2	1.04	0.350
5-21 days	Control	Zeolite	Granite	MSE	P-value
Weight gain g	882	921	893	47.6	0.2298
Feed intake g	1201	1235	1215	53.2	0.3775
Feed/gain	1.36	1.34	1.36	0.040	0.4626

¹85%starter diet +15% whole wheat; ²square of mean square errors.

4.2 Dissection data

The data related to gizzard characteristics are illustrated in Table 2.

At 13 days of age, gizzard weight increased ($p<0.05$) when birds given granite grit. Gizzard content and relative content were significantly heavier in birds fed granite grit than birds fed zeolite. No significant difference in gizzard weight and gizzard

contents was observed between all treatments both on day 18 and 21.

A higher ($p>0.05$) amount of grit found in the gizzard from birds given granite than zeolite at each dissection day. The pH of the gizzard contents was not significantly affected by grit addition at the same age. However, the pH of gizzard content was increased from 13 to 18 days of age and become lower at 21 days of age when the diet including 15% whole wheat, regardless the with or without grit. Relative gizzard weight decreased with increased body weight from 13 to 18 days of age, regardless the treatments. However this tendency was not detected in granite-fed birds and control birds from 18 to 21 days of age.

Table 2. Gizzard content weight, gizzard weight, grit in gizzard and gizzard pH from different days of age

13 day	Control	Zeolite	Granite	Sq. MSE	p-value
gizz. ¹ content g	6.6 ^{ab}	6.1 ^b	8.5 ^a	1.96	0.0082
Empty gizz. Weight g	10.5 ^b	11.0 ^{ab}	11.7 ^a	0.97	0.026
R gizz. Content ¹ %	1.34 ^{ab}	1.24 ^b	1.63 ^a	0.394	0.036
R gizz. Weight ² %	2.14	2.21	2.26	0.226	0.4786
Bird body weight g	495	497	518	31.5	0.16
Grit in gizzard g	-	0.86 ^b	3.13 ^a	0.575	<. 0001
Gizzard pH	3.1	3.5	3.4	0.59	0.328
18 day	Control	Zeolite	Granite	Sq. MSE	p-value
gizz. ¹ content g	8.1	7.1	8.1	5.11	0.8932
Empty gizz. Weight g	14.0	14.0	13.8	2.11	0.9554
R gizz. Content ¹ %	0.89	0.79	0.91	0.559	0.8728
R gizz. Weight ² %	1.56	1.58	1.55	0.256	0.9528
Bird body weight g	898	898	894	73.0	0.9902
Grit in gizzard g	-	0.09 ^b	1.64 ^a	0.935	0.0002
Gizzard pH	3.3	3.6	3.6	0.80	0.80
21 day	Control	Zeolite	Granite	Sq. MSE	p-value
gizz. ¹ content g	8.6	9.6	10.2	2.51	0.3638
Empty gizz. Weight g	16.5	16.8	16.7	1.90	0.9213
R gizz. Content ¹ %	0.82	0.86	0.94	0.212	0.4170
R gizz. Weight ² %	1.59	1.52	1.56	0.172	0.6632
Bird body weight g	1043	1107	1070	104.7	0.3722
Grit in gizzard g	-	1.55 ^b	2.86 ^a	1.12	0.0001
Gizzard pH	3.0	2.8	2.9	0.48	0.6643

¹gizzard; ² empty gizzard weight expressed as % of body weight; ³ gizzard content weight expressed as % of body weight. ⁴ square of mean square errors; ^{abc} means in a row with same superscript are no significant difference ($p > 0.05$)

Table 3 shows that approximately 60% of each type of grit excreted from birds during 5 to 18. The highest amount of grit excreted during 5 to 11 days, which is 39% for zeolite and 45% for granite respectively. A significantly higher amount of zeolite disappeared than granite from 5 to 18 days of age, which represents the proportion of grit neither found in gizzard nor found in excreta.

Table 3. Grit consumption; grit disappearance and grit passage

	Zeolite	Granite	Sq. MSE ⁴	p-value
Grit consumption ¹ g	37	37	0.91	0.9114
Grit disappearance ² %	34 ^a	11 ^b	11.3	<. 0001
Grit passage ³ 5-11 %	39	45	8.5	0.1540
Grit passage 11-13 %	18 ^a	12 ^b	6.2	0.0065
Grit passage 13-18 %	7 ^b	10 ^a	4.2	<. 0001

¹ mean amount of grit consumed by each cage from same treatment; ² grit amount found in excreta expressed as percentage of total grit consumption; ³ grit amount except from found in excreta and gizzard expressed as percentage of total grit consumption; ⁴square of mean square errors; abc Means in a row with same superscript are no significant difference ($p>0.05$)

4.3 Excreta particle size distribution

The excreta particle size distribution from 18 to 21 days of age is presented in table 4. Despite the fact that whole-wheat kernels with a size larger than 1.4mm, the percentage of excreta particle size larger than 1.4mm were not decreased ($p>0.05$) by grit addition. Also, the particle size fraction smaller than 0.2 mm was not increased when the birds given grit. The proportion of excreta particles between 1.4-0.8mm increased ($p<0.05$) in granite group and the percentage of particles between 0.5-0.2mm increased ($p<0.05$) in zeolite group.

Table 4 Excreta particle size distribution¹ from 18 to 21 days of age²

Size	Control	Zeolite	Granite	Sq.MSE	p-value
> 1.4 mm %	19	21	17	6.5	0.1626
1.4-0.8 mm %	9 ^b	8 ^b	13 ^a	3.4	0.0029
0.8-0.5 mm %	10	10	10	3.4	0.9269
0.5-0.2 mm %	14 ^b	22 ^a	15 ^b	4.2	<0.0001
< 0.2 mm %	48	40	45	14.5	0.1540

¹excreta particle size distribution expressed as proportion of entire faeces as dry matter bases; ² 15%whole wheat included in the diet; ³ square of mean square errors. The variance of the means value; ^{abc} means in a row with same superscript are no significant different ($p>0.05$)

Table 5 shows that birds in grit group tend to consume higher ($p=0.057$) amount wheat than the control group in 5 hours.

The amount of whole wheat presented in the crop was significantly ($p<0.05$) higher in

zeolite group than control group. No significant differences in whole wheat presented in the gizzard and small intestine between all treatments. No significant ($p>0.05$) difference between all treatments in whole wheat presented in the excreta. No significant ($p>0.05$) difference was observed in the amount of whole wheat passed through the crop and gizzard between all treatments. Similar ($p>0.05$) amount of whole wheat ground in the gizzard in all treatments.

4.4 Whole wheat in different digestive segments

Table 5 amount of whole-wheat consumption, whole wheat passed crop and gizzard and amount of whole wheat grinded

	Control	Zeolite	Granite	Sq.MSE	p-value
Whole wheat consumption DM g	16.1	27.4	21.5	7.52	0.0571
Whole wheat in crop DM g	2.3 ^b	7.6 ^a	4.4 ^{ab}	3.04	0.0101
Whole wheat in gizzard DM g	2.6	2.9	1.3	1.02	0.0694
Whole wheat in small intestine DM g	0.1	0.1	0.1	0.15	0.7550
Whole wheat in excreta DM g	0.2	0.1	0.3	0.46	0.7320
Whole wheat passed the crop DM g	13.8	19.9	17.0	5.43	0.2736
Whole wheat passed the gizzard DM g	11.2	17.0	15.8	4.95	0.1996
Whole wheat grinded DM g	10.8	16.8	15.4	5.00	0.1855
Wheat grinded/wheat passed crop %	75	85	87	10	0.1939

¹ amount of whole wheat consumed by per bird expressed as dry weight; ² amount of whole wheat leaving crop expressed as dry weight; ³ amount of whole wheat leaving gizzard expresses as dry weight; ⁴ amount of whole wheat grinded in the gizzard expressed as dry weight; ⁵ amount of whole wheat grinded in the gizzard calculated as percentage of whole wheat passed through the crop; ⁶ square of mean square errors; ^{abc} means in a row with same superscript are no significant difference ($p>0.05$)

5. Discussion

5.1 Effect of grit on grinding and excreta particle size distribution

From the experiments herein recorded, it is apparent that bird's gizzard can grind

whole-wheat kernels efficiently without grit. This result is in accordance with Hetland et al. (2002); Svihus et al. (1997a) who suggested that broiler chickens are able to grind whole cereals efficiently. In the current study, we observed that a similar proportion of whole wheat grinded down between all treatments. Furthermore, we found very few whole-wheat kernels in the excreta although the birds fed without grit. This observation corresponds with Svihus et al. (1997b) who reported that very few whole barley kernels found in the excreta from 16-d-old broilers fed 700g/kg whole barley in the diet.

Since the gizzard is the only organ that breaking down feed, excreta particle size distribution could represent the extent of grinding ability of the gizzard. In this study, we observed that the proportion of the excreta particles that below 0.2 mm, was not increased by grit. Also, the excreta particles larger than 1.4 mm were not decreased by grit addition. However, particles with a size between 0.5-0.2mm and 1.4-0.8mm were increased by zeolite and granite, respectively. The most important evidence that could demonstrate grinding ability is the amount of particles smaller than 0.2mm. Therefore we concluded that broiler chickens gizzard has the ability to grind whole grains without grit.

On the other hand, according to Hetland et al. (2003), providing grit to broiler chickens fed on 200g/kg whole wheat diet result in an increasing amount of very small duodenal particles below 40 μ m compared to birds fed same level whole wheat without grit. In their study, the gizzard weight was also increased by grit and the grit in the gizzard was heavier than our observation. However, in the same study, although the gizzard weight was increased due to oat hulls the duodenal particles were not affected.

5.2 Effect of grit on performance of broilers

In the current study, neither growth rate nor feed conversion efficiency was improved

by grit. This result is in line with Kennard and Chamberlin (1959), Hollister (1991) and Garipoglu et al. (2006) who concluded that chickens does not benefit from grit.

On the other hand, Balloon and Phillips 1956 observed that feeding insoluble grit to laying hens improved weight gains and feed conversion efficiency of chickens, regardless the feed form (mash and grain or all mash). However, the laying hens were most beneficial when whole grains are part of the diet. Also, Heuser and Norris (1946) reported that the addition of granite grit to a mash diet did not influence chick weight, but there seemed to be some improvement in feed efficiency.

In this study, during 11 to 13 days of age, birds in fed grit group seemed to have a higher ($p=0.05$) feed intake than the control birds. It may to due to the heavier gizzard observed at 13 days of age in grit-fed bird. As mentioned in the literature review, the increase in gizzard weight is due to more extensive grinding action to reduce the larger particles. This indicates that heavier gizzard due to grit stimulation may contract fast and frequently than gizzard without girt. Engberg et al. (2002) reported that pellets moistened and dissolved fast and easy to expose microstructure and thus quickly passes through the gizzard into small intestine. The stronger contraction of the gizzard then may led to dissolved pellets passing through the upper digestive tract more faster and the gizzard emptying fast. And thus result in increased in food intake.

From previous reports, it would be expected that grit in the diet would exert a greater influence on feed utilization when whole grain was fed. Scott and Heuser (1957) found that insoluble grit improved weight gain and feed efficiency of broilers when the diet including whole grain. In the present study, although whole wheat introduced from 18-21 days of age, the live performance was not affected by addition grit. Therefor our result failed to support the hypothesis that chickens achieve better feed utilization due to grit when whole wheat included in the diet.

This observation was in accordance with the result reported by Bennett et al. (2002a)

and Bennett and Classen (2003) who studied the influence of whole barley and grit on turkey and hens. In their study, the authors observed that feeding grit had no beneficial effect on the live performance of birds fed whole barley or mash diets.

Similar results also have been reported by Bennett et al. (1995) and Svihus et al. (1997a) who reported that feeding grit has no beneficial effect on body weight and feed conversion of birds when the diet including whole grain.

5.3 Effect of grit on gizzard development

In the current study, birds receiving granite grit developed heavier empty gizzard than birds fed no grit at 13 days of age, but no effect on relative gizzard weight. Our observation was partly in line with previous results that reported by Gionfriddo and Best (1999), Garipoglu et al. (2006), Itani (2015). In their studies, researchers detected that domestic birds given access to grit often developed heavier gizzards than those birds feeding without grit. The increase in gizzard weight has been expected when birds receiving grit. It would appear more grinding action in the gizzard when the hard texture of grit presented in the gizzard. Consequently, birds will develop stronger and heavier gizzard. However, it is not the case in zeolite grit. According to Smith and Macintyre (1959) the texture of grit such as hardness, durability and insolubility influence the effectiveness of the grit as a grinding agent. Zeolite girt is softer than granite girt and the original size we used in the feed was smaller than granite. Therefore the soft texture of this type of stone may be the explanation of the lack of stimulating effect on gizzard.

Beside of this reason, a significant higher amount of granite found in the gizzard than zeolite at this age. This small amount zeolite might not enough to stimulate the gizzard muscle to develop heavier than those no grit group.

However, grit effect on gizzard weight was not observed at 18 days of age. The small

amount of grit presented in the gizzard may partly explain the lack of stimulating effect of grit on gizzard. We observed that birds excreted almost 50% of grit in the faeces from 11 to 18 days of age and consequently the grit amount found in gizzard was much lower than that found in gizzard at 13 days of age.

The small amount of grit presented in the gizzard also may be the reason that we observed similar empty gizzard weight and relative gizzard weight at 21 days of age. Although each bird has been given 3g of grit during 18 to 20 days, the grit presented in the gizzard at 21 days of age was still not as much as 13 days of age.

Alternatively, feeding whole wheat during 18 to 21 days of age may be the reason that grit had a lack of effect on gizzard weight. Svihus et al. (1997a) has been studying the effect of the whole, rolled and ground barley with and without grit on the performance of broilers. In their research, the authors indicated that grit addition did not affect relative gizzard weight, but the larger gizzards observed when broilers fed the diet including whole barley compared to those fed ground barley. It may indicate that the whole wheat as a structural component could appear stimulating effect on gizzard. In order to break down the whole grains the gizzard contracts harder and thus gizzard muscle develop enough to grinding down whole wheat for a sufficiently small size to pass through the gizzard.

In contrast to coarse ground or whole grain feed, pelleted diet could dissolve rapidly after consumption and exposed the fine microstructure in the gizzard (Svihus 2011), which is no stimulating effect on gizzard. Mash diet or coarser ground diet increased in relative gizzard size (Engberg et al. 2002; Svihus et al. 2004). Furthermore, studies also have shown that structural component such as oat hulls, wood shaving result in enlargement of the gizzard size (Amerah et al. 2009; Biggs & Parsons 2009; Hetland & Svihus 2001; Hetland et al. 2003; Plavnik et al. 2002).

The similar gizzard weight observed between all treatments in the current study may

partly explain the lack of effect on broiler performance. However, Itani (2015) and Garipoglu et al. (2006) found that live performance was no significant difference between birds fed grit and without grit, although in their experiment heavier gizzard has been detected. Therefore it may indicate that the gizzard weight maybe not a determining factor for improvement in growth rate or feed utilization. In addition, Scott and Heuser (1957) also concluded that gizzard or relative gizzard weight were not conclusive criteria for an improvement of feed utilization.

5.4 Effect of grit on AME

In the current study, AME was not affected by grit addition, either when the diet with or without whole wheat. This result not in line with the result reported by Smith and MacIntyre (1959) who reported that grit feeding enhanced the ME contents.

Mcintosh et al. (1962) studied the interaction of whole, ground and pelleted grain with grit stone. In their experiment, the authors found that the ME contents of wheat and barley were not increased by feeding of hen-size grit. However, the feeding of grower-size grit increased the ME contents of both ground, pelleted and whole grains and the effect of grit feeding on ME was greater with whole grain than with ground and pelleted grains. Therefore it indicated that grit has some other actions in addition to grinding the grain in the gizzard. Furthermore, Smith and MacIntyre (1959) indicated that grit improves the digestibility of feeds and this improvement is increased as the degree of coarseness of the feed consumed is increased. Balloun and Phillips (1956) and Smith and MacIntyre (1959) have presented the data which indicated that a slightly better feed efficiency, and digestibility exhibited by sand which has smaller particle size than hen-size insoluble grit in their experiment. Which may indicate that particle size also influence the effect of grit in the gizzard.

The lack of a significant effect of grit feeding on AME may have been due to the fact

that a small amount of grit used in the current study, which is in contrast to the greater quantities grit used in McIntosh et al. (1962) experiments where the grit was up to 58 g per birds.

6. Conclusion

From current experiment it showed that grit supplementation did not impair nor improve broiler performance compared to control treatment. It could be concluded that modern broiler chickens are able to grind diets containing whole-wheat grains efficiently without grit. It is therefore possible that whole wheat can replace part of ground wheat without a negative effect on performance.

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