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The Effect of Granite Grit on Broiler Chickens Performance and Gizzard Development

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Sammendrag

For å undersøke effekten kråsstein på slaktekyllingens ytelse og kråsutvikling, ble en omfattende studie utført ved Senter for Husdyrforsøk, ved Norges Miljø- og Biovitenskapelige Universitet i Ås, Norge. 252 dag gamle hannskaltekyllinger ble oppfostret på kuttflis i fire forskjellige bur. De fikk tilgang til kommersielt startfôr, og hadde fri tilgang til både fôr og vann under hele forsøket (med unntak av to sulteperioder). På dag 5, ble 192 fugler fordelt i grupper på fire i 48 vaktelbur som ble i tillegg delt in i fire behandlinger; kontroll, zeolitt kråsstein, granitt kråsstein og marmor kråsstein. De tre kråssteingruppene fikk tilgang på 9.5 gram kråsstein/fugl fra dag 5 til dag 11. På dag 11, ble startfôret byttet over til kommersielt vekstfôr. Startfôr med kråssteinrester ble spart for å beregne kråssteinkonsum. På dag 13, 18, 21 og 22 ble en fugl fra hvert bur drept med et kranialt slag etterfulgt av et cervikal dislokasjon. Kråsen ble veid både full og tom, og kråsinnholdet ble fryst umiddelbart. Tarmene ble også dissekert ut og fryst. Hele kroa ble kun dissekert ut og fryst på dag 21 og 22. På dag 18 fikk fuglene en diet som bestod av 15% startfôr og 85% hel hvete, og fikk i tillegg 1 gram kråsstein/fugl på dag 18, 19 og 20. På dag 20 og 21 fastet fuglene i 10 timer før de fikk tilgang på fôr igjen for å undersøke passasjehastighet. Ekskrementer ble samlet sammen for dag 5-11, 11-13, 13-18 og 18-21, samt ble vektøkning og fôrkonsum også registrert for disse periodene. Partikkelfordeling av ekskrementer og kråsstein funnet i ekskrementer for disse periodene ble kalkulert. Mengde kråsstein tilbakehold i kråsen og dens partikkelfordeling ble kalkulert for alle dissekeringsdagene.

Denne masteroppgaven fokuserer på granittbehandlingen. Det ble ikke funnet noen signifikant effekt av granitt sammenlignet med kontrollen for noen ytelsesparameterne. Det var signifikante forskjeller for passasje og partikkelfordeling i fordøyelsessystemet mellom granitt kråssteinen og de andre kråssteinbehandlingene. En signifikant høyere mengde granitt ble tilbakeholdt i kråsen sammenlignet med de andre kråssteinbehandlingene. Det var ingen signifikante forskjeller for kråsutvikling mellom granitt og kontroll, med unntak av tom kråsvekt ved dag 13 som var tyngre for granitt. Generelt var konklusjonen at granitt kråsstein verken forbedret eller forverret ytelsesparametere eller kråsutvikling for slaktekylling i denne studien.

Nøkkelord: Kråsstein – Granitt – Ytelse – Tilbakeholdt – Kråsutvikling – Partikkel størrelse

Abstract

To investigate the effect of grit on broiler chickens performance and gizzard development, a comprehensive study was performed at the Animal Production Experimental Centre, at the Norwegian University of Life Sciences (NMBU), Ås, Norway. 252 day old male broiler chickens were raised on wood shavings in groups of four in different pens. They were given commercial starter feed, and had *ad libitum* access to both feed and water for the entire experiment (with two starvation periods). At day 5, 192 birds were divided into 48 quail cages with 4 birds in each cage, the quail cages were further divided into four treatments; control, zeolite grit, granite grit and marble grit. The three grit groups were given a total of 9.5 grams grit/bird to their respective treatment from day 5 to 11. On day 11, the starter feed was changed to commercial grower feed. Stater feed residues with potential grit residues were saved to calculate grit consumption. On day 13, 18, 21 and 22, one bird from each cage was killed with a cranial blow followed by a cervical dislocation. The birds' gizzards were weighed full and empty, and gizzard content was immediately frozen. Small intestines were also dissected out and immediately frozen. The entire crop was only dissected out on day 20 and 21. On day 18, the birds got access to a diet consisting of 15% whole wheat and 85% starter feed, and were given 1 gram grit/bird on day 18, 19 and 20. On day 21 and 22 the birds were starved for 10 hours before getting access to feed again to investigate passage rate. Excreta were collected and pooled together for day 5-11, 11-13, 13-18 and 18-21. Weight gain and feed consumption were also registered for these periods. Particle distribution of faeces and grit recovered in faeces were calculated for the different collection periods. For all the dissections days, grit retention in gizzard and particle size distribution of grit in retained in gizzard were calculated.

This master thesis focused on the granite treatment. There were found no significant effect of granite grit compared to the control on any performance parameters. There were significant differences between granite passage and distribution in the digestive tract other grit treatments. A significantly higher amount of granite were retained in the gizzard compared to the other treatments. There were no significant differences on gizzard characteristics between the granite treatment and control, except for a higher empty gizzard weight at day 13. Overall, the conclusion were that amount of given granite grit did not have any significant effects on broiler performance or gizzard development in this study. Granite did neither improve nor impair performance or gizzard development in broiler chickens.

Keywords: Grit – Granite – Performance – Retention – Gizzard development – Particle size

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1 Introduction

The poultry industry is the largest animal production in the world and make up approximately 80% of all livestock in the world (FAO 2013a). The products it creates are meat and eggs, which consists of high-quality protein, vitamins and minerals (FAO 2013b). Numbers from 2013 estimate that there were 20 billion chickens and 7 billion eggs produced (FAO 2013a), and one can estimate that the numbers are higher in 2016. The fat composition of the chicken meat is considered healthy, with a general low content of fats and a high proportion is unsaturated fats. From a religious point of view, poultry products are perfectly fine to eat. The general size of the products are also beneficial as for instance in undeveloped countries, were there are limited opportunities to store or chill animal products to avoid spillage, making one chicken is a well sized meal for one family (FAO 2013b).

Costs related to poultry feed production can easily make up to 70% of the total production costs (Svihus 2002). Many factors influence the cost and energy use related to feed production, i.e. grinding method will influence the energy consumption and thus the production costs. Reece et al. (1985) found that the roller mill required 14.5% less energy compared to the hammer mill, whereas Deaton et al. (1989) found no significance effect of hammer mill versus roller mill on layers' performance. Svihus et al. (2004) concluded that coarse grinding of wheat does not have a negative effect on broiler performance. It is therefore desirable to reduce costs by for instance usage of whole grains, as home-grown whole cereals reduces transportation and processing cost or simply by using coarse grinding. Chickens are well adapted to grind whole grains or coarsely ground feed without the addition of grit (Svihus et al. 1997). Amerah et al. (2007) concluded that coarsely ground wheat in mash diets had no negative effect on broiler performance, and had a positive effect on feed intake, weight gain and feed:gain. Thus, it is beneficial to use coarse grinding with respect to energy savings in the feed processing costs.

Since birds does not have any teeth, the gizzard functions as a grinder. The main function of the gizzard is to grind the feed particles down into a certain size, before entering the duodenum and rest of the intestines. It is known that the particle size of the feed affects the gizzard development, and fine particles will reduce gizzard development (Nir et al. 1995). Whereas inclusion of structural components such as oat hulls, whole wheat, wood shavings and grit will increase the gizzard size and weight (Biggs & Parsons 2009; Bjerrum et al. 2005; Hetland et al. 2003).

It is postulated that grit will increase the grinding of grain in the diet, and thus increase the surface of the feed particles (Jones & Taylor 1999) which hopefully leads to an increase in digestibility. It is also postulated that grit will also improve the development of the gizzard muscle, and increase the grinding ability and therefore the digestion of nutrients.

A comprehensive study was performed at the Animal Production Experimental Centre, at the Norwegian University of Life Sciences (NMBU), Ås, Norway, the study focused on different grit supplementation for broiler chickens. The hypothesis in this master thesis was that the addition of grit would stimulate the gizzard and thus increase the birds' performance. Also due to gizzard stimulation, and thus an increased ability to grind, it was also hypothesized that the grit treatment would have a greater ability to utilize a diet that consisted of whole wheat. This master thesis will focus on the granite grit treatment and its effect on performance and gizzard development. The thesis does not focus on the other grit treatments or passage rate.

2 Literature review

2.1 Avian digestive system

The avian digestive system has many similarities to other monogastric animals' digestive system, it uses the same principles as ingesting feed, moisturizing the material, breaking down the material to smaller particles, acidifying the particles and further breakdown by the use of endogenous enzymes (Svihus 2014).

Birds are natural seeking and curious beings and one out of three pecks results in feed ingestion (Svihus 2002), where the feed is immediately swallowed and either stored in the crop or directly passed further down the digestive tract. The crop is a very flexible organ on the esophagus, which functions as a feed storage. When the birds are fed *ad libitum*, the storage capacity is hardly utilized as the birds get used to continuous access to feed and thus not require to store feed in the crop. Birds that are used to intermittent feeding will utilize the storage capacity to a higher extent. Feed that is stored in the crop will be moisturized, but will not be exposed to any enzyme secretion or other digestive process (Svihus 2014). The crop is followed by the proventriculus and ventriculus, which will be further described in the next paragraph. The small intestines functions as the main site for digestion and nutrient absorption. Bile and pancreatic juices are secreted from the gall bladder and pancreas and aid the digestion of nutrients that are absorbed over the intestinal wall. Poultry have a pair of ceca, which are emptied approximately two times a day, which means that the retention time is very long (Svihus 2014). The main function of the ceca is to absorb electrolytes and to some extent water, and possible the recirculation of retinal nitrogen (Svihus 2014).

One difference between birds and other animal species is the lack of teeth. To compensate for this the bird has a special two-part stomach system, a glandular and muscular stomach. The feed will first enter the proventriculus, the glandular stomach, where the feed is moisturized and mixed together with hydrochloric acid, pepsinogen and mucus. Directly after the proventriculus is the ventriculus, the muscle stomach, more commonly known as the gizzard. The gizzard mixes and grinds the moisturized, acidified feed material and functions as teeth does in other species. The muscles consists of strongly myelinated smooth muscles (Svihus 2011), that grinds the particles by contracting the muscle walls and causing crushing and abrasion forces to break down the particles. Grit can enforce this process, as it works as a grinding agent. The walls of the gizzard have a koilin layer which is hardened by low pH (Svihus 2011), which is caused by the hydrochloric acid secreted in the proventriculus, and thus also contribute to the grinding process. The koilin layer, a carbohydrate-protein complex, is continuously renewed as it is worn down (Svihus 2011). As particles decrease in size, the surface:volume ratio increases and thus the digestibility potential in increases due to a larger area available for the digestive enzymes.

As described in *Avian Biology 2*, Chapter 6, “Digestion and the digestive system” by Ziswiler and Farner (1972), the gizzard has several functions:

- Storage and filtering: Feed will be stored in the gizzard until it is ground down to a suitable size for further digestion in the small intestines thus the gizzard functions as a filter when retaining the large particles until they have been broken down.
- Preliminary acid proteolytic digestion: Hydrochloric acid secreted in the proventriculus will moist the feed particles and be mixed together in the gizzard.
- Mechanical digestion: The muscle contractions in the gizzard will increase the mixing of feed and hydrochloric acid by rubbing the feed particles together causing them to break and reduce in size. There are several interactions between type of feed particle, structure of feed particle, muscle development of the gizzard, koilin layer, retention time in the gizzard and grit (and type of grit, i.e. insoluble versus soluble).
- Passage: Contractions in the gizzard will either push material back to the proventriculus for additional moisturizing of hydrochloric acid, or through the pylorus and into the duodenal part of the intestines. It is possible for material to be pushed back from the duodenal part and back into the gizzard (Clemens et al. 1975), and thus increasing the digestion due to interaction between gizzard contractions, grinding, mixing and digestive enzymes from the duodenum.

2.2 Feed structure

The main difference when it comes to dry animal feed is mash versus pelleted diets and when feeding different kinds of feed, it is important to both look at the macro- and microstructure of the feed. The macrostructure is the pellet itself, while the microstructure is the structure of the components in the pellet or mash, i.e. whole grains or the size of the ground materials. These particles have to be a given size before leaving the gizzard through the pylorus and entering the duodenum. The gizzard is capable to grind both fine and coarse feed particles down to a similar duodenal particle size distribution, regardless of the original size (Amerah et al. 2008).

The microstructure can be divided into everything from fine to coarse particles, with the addition of whole grains, hulls, grit or other structural components. Pelleted feed compared to mash diets will increase the feed intake and thus the weight gain and improve feed conversion ratio (Amerah et al. 2007; Chewning et al. 2012; Engberg et al. 2002). Dahlke et al. (2003) found a linear growth in increased gizzard weight and increased particle size in both pelleted and mash diets, which implies that the microstructure highly affects the gizzard development. Although it is desirable to have a high weight gain, it is also important to consider the effect the growth has on the bird itself; birds that are susceptible for ascites, a specific type of congestive heart failure in broiler chickens, will most likely develop this disease when they achieve too high weight gains (Arce-Menocal et al. 2009). It may thereby be advisable to find a combination between proper, healthy weight gain and feed particle form and size for broiler chickens.

Microstructure is also very important in how the gizzard processes the material. Moore (1999) found that, when giving geese different cut length of grass, the particles leaving the gizzard and entering the intestinal tract were rather constant, but the processing of the original particle size was different; 2 mm particles, compared to the 10 mm particles, were not always broken down as finely. It can be concluded that the gizzard grinds material down to a specific size before leaving the gizzard, but the interaction mechanisms between macro- and microstructure is different. Parsons et al. (2006) investigated how the particle size of corn in mash diets and the hardness of pellets affected broiler performance. They concluded that feeding medium to coarse particle size had a positive effect on nutrient digestion, but the increased gizzard growth for the birds that ate the coarse diet may had a negative effect on breast growth as the maintenance requirement for gizzard increased. Hard pellets did also have a beneficial effect on nutrient digestion. These findings implies how the macro- and microstructure affects factors like performance and digestibility.

Numerous studies have shown an increase in gizzard weight when the gizzard is stimulated by structural components such oat hulls, whole wheat or wood shavings (Amerah et al. 2009; Biggs & Parsons 2009; González-Alvarado et al. 2007; Hetland & Svihus 2001; Hetland et al. 2002; Hetland et al. 2003; Jones & Taylor 2001; Svihus et al. 1997). Due to these findings a general conclusion is that gizzard development is strongly influenced by particle size. Amerah et al. (2009) suggested that the inclusion of coarse fibers has a beneficial effect on broiler performance due to an improved feed:gain ratio when corrected for insoluble fibers, which was also found by Hetland et al. (2003). A well developed gizzard is considered positive for further digestion in the intestines (Hetland & Svihus 2001; Svihus 2011).

As structure has a positive effect on gizzard development it is logical to assume that this also will have a positive effect on the birds' health status. Bjerrum et al. (2005) found a decrease in gizzard pH and *Salmonella* Typhimurium bacteria when whole wheat was included in the diet. This supports the theory that a well developed gizzard results in a better general health status for the birds as the feed particles are retained in the gizzard and exposed to hydrochloric acid for a longer time, which results in fewer pathogenic bacteria entering the small intestines. As well, a longer retention time increases the digestion of the feed for the bird, and thus decreases potential substrate for harmful pathogens. Biggs and Parsons (2009) also concluded similarly; feeding whole grains might improve the nutrient digestibility while interacting with the increased gizzard weight and thus have a positive effect on the intestinal environment as less undigested nutrients is available for pathogenic bacteria. Hetland et al. (2002) concluded that the gizzard have a high ability to grind whole cereals, even without the addition of grit, which will increase the digestibility and leave less substrate for harmful microorganisms. Evans et al. (2005) found that laying hens had significantly lower coccidiosis oocyte output when fed whole wheat rather than ground wheat. They concluded that it is beneficial, both on health and economy factors, to include whole wheat in the diet. These results are in contrary to findings by Waldenstedt et al. (1997), who did not find any effect of feeding whole wheat, even with or without grit addition, on performance for birds infected with coccidiosis bacteria *E. maxima* or *E. tenella*.

Structure may also effect the passage rate, a more rapid rate have several effects; increased feed intake, reduced time for digestion and thus reduced utilization of nutrients for both the bird itself and microorganisms in the intestines. Hetland and Svihus (2001) found a tendency for faster passage rate with the inclusion of coarsely ground oat hulls in a mash diet compared to a finely ground oat hull mash diet. However, Svihus et al. (2002) did not find a significant difference in passage between ground and whole wheat in a pelleted diet. It is logical to assume that larger particles will be retained for a longer time before leaving the gizzard, as they need to be broken down to a given size which takes more time and energy than smaller particles. The gizzard also has a selective ability to retain larger particles, and Rougière and Carré (2010) found results indicating that chickens bred for a high digestion efficiency will have a longer mean retention in the proventriculus and gizzard time than chickens selected for a low digestion efficiency. A longer retention time will increase effects of interactions between factors like grinding, acidification, mixing and thus the digestibility potential will increase. The inclusion of fiber in the diet decreased the differences between the two breeds, indicating that structure are important for passage from the gizzard and further into the intestines.

As mentioned, duodenal particle size distribution will be similar for both fine and coarse feed particles (Amerah et al. 2007; Amerah et al. 2008), supporting the theory that the gizzard grinds the particles down to a given size before the particles are let through the pylorus. There is an interaction between both gizzard and pylorus, as the gizzard grinds down the particles and the pylorus function as a filter and retain too large particles until they are small enough.

2.3 Grit

Gritstone, gastrolith, stomach stone, grit or gizzard stone are all synonyms of stones of various composition that the bird ingest and store in the gizzard to aid the grinding process in the gizzard and thus digestion of nutrients. Grit may also be a source of minerals, i.e. calcium, which are very important for laying hens and growing chickens (Gionfriddo 1994). No types of grit function as an energy source, but rather as an energy dilution in the diet. Birds will voluntary eat grit and use it as a grinding aid in the gizzard and to maximize the grinding of material, it is presumed by Beer and Tidyman (1942) to be necessary for gallinaceous birds, and if grit is not available, birds will consume hard seeds as a substitute.

Grit can come from soluble and insoluble sources. Limestone and oyster shells are commonly used as a soluble grit, and may to some extent dissolve due to the low pH in the proventriculus and supplement the bird with minerals, especially calcium supplement for layers. Marble grit is also a soluble grit source which have basically the same chemical formula than limestone. Granite, quartz, silica, feldspar and zeolite are insoluble grit, and are more resistant of the dissolving pH secreted in the proventriculus, thus having a longer retention time in the gizzard. McIntosh et al. (1962) states *"it is generally accepted that the feeding of grit to poultry is economically worthwhile."* Smith and MacIntyre (1959) also stated that grit feeding would have a positive effect on the bird. As of today, grit is not widely used in industrial poultry production.

Branion and Heuser (1960) have extensively reviewed the usage of grit for poultry from studies from 1785-1960. The review claims different effects of grit, such as; improved weight gain and feed conversion ratio (Balloun & Phillips 1956), improved weigh gain in combination with ground and whole grain milo (Balloun (1959) in Branion & Heuser, *Grit for poultry: Bibliography and abstracts*), more healthy gizzard muscles (Brook (1957) in Branion & Heuser, *Grit for poultry: Bibliography and abstracts*), improved digestibility coefficients for protein, fat and other carbohydrates, but not for crude fiber (Danilova et al. (1933) in Branion & Heuser, *Grit for poultry: Bibliography and abstracts*). There are also, reviewed by Branion and Heuser (1960), documented no or little effects by addition of grit; Same efficient utilization of feed with or without granite grit (Bethke & Kennard 1926) and high culling rate and retarded egg production when the birds had *ad libitum* access to limestone (Blount (1948) in Branion & Heuser, *Grit for poultry: Bibliography and abstracts*). Grit may even have a double effect; Bird et al. (1937) stated that grit feeding or a coarse ration may have a preventative effect on crater lesions, but the grit itself can have a bad effect on crater lesions if the lesions already are present. Riedel (1950) only found a numerical improvement in weight gain when broilers had *ad libitum* access to granite grit, but it was not significant compared to the control. Fuller (1958) compared no grit, granite grit and marble grit and found that even when grit consumption were almost equal, the granite group had a slightly higher body weight and better feed:gain than the marble group even though the differences no were significant. The marble group also had a higher mortality than the granite and no grit group.

Jones and Taylor (1999) found inconsistent and inconclusive results when feeding broilers *ad libitum* with limestone or granite grit. According to their results, grit might improve growth and performance, but type of grit seemed to be of more influence. Garipoglu et al. (2006) did not find any improved performance on broilers when offering *ad libitum* insoluble granite grit, but they did see a tendency for lower feed intake. Bennett et al. (2002) concluded that insoluble grit had no effect on whole grain diets for turkeys. Older literature by McIntosh et al. (1962) showed an increase in metabolizable energy (ME) when feeding grower-sized grit instead of hen-sized grit, which shows the importance of interactions between grit and several factors like shape and size of grit. These factors interacts with the relative weight and development of the gizzard as young birds, for instance, broilers will not have the same ability to utilize large grit particles to the same extent as older birds, as for instance layers. McIntosh et al. (1962) also postulated that the grinding action of the grit in the gizzard may facilitate the enzymatic digestion as grinding presents a greater surface:volume ratio, and possible the retention time increases when grit are present and thus allow a more complete digestion. There is also a combination effect between insoluble and soluble grit; Scott and Heuser (1957) found improved results regarding growth, egg production and feed utilization for chickens and turkeys when feeding insoluble grit compared to birds fed no grit or only calcium grit. One interesting fact they also discovered is that the development of the gizzard would reach the same maximum point regardless treatment, but the grit group would achieve this point earlier in the development than the no grit group. Therefore, by adding grit to the diet, the bird might have an improved growth of the gizzard at an earlier stage in life and thus have a positive effect on further development. Spencer and Jenkins (1963) found an increased gizzard weight that was highly significantly correlated with retained grit in the gizzard, the increase in gizzard weight also resulted in an increased gizzard size due to grit feeding.

Additionally, as the feed particles, the grit may also be ground down and passed further trough the digestive tract. Vance (1971) recovered approximately ¼ of the consumed grit in faeces when feeding Neoga or Sibley grit, unlike Korschgen et al. (1965) who found only traces limestone grit in faeces. Vance (1971) also compared the mineral composition in grit as fed, in the gizzard and in the faeces, and saw that several minerals decreased in concentration to a larger extent from as fed to gizzard than from gizzard to intestines, indicating that ions are to a larger extent dissolved in the gizzard rather than further down in the digestive tract. This may also explain why Korschgen et al. (1965) did not find any limestone in the intestines as it easily dissolves in the gizzard due to hydrochloric secretion in the proventriculus. Anderson and Stewart (1973) found that the concentration of several ions in grit in the gizzard were less than the concentration of the grit in the soil for pheasants, and stated that this was possible due to digestive activity in the gizzard. This supports the theory that grit have a role as a mineral source for birds.

Since the gizzard has an increased development with grit, structural components or both (Amerah et al. 2009; Biggs & Parsons 2009; González-Alvarado et al. 2007; Hetland & Svihus 2001; Hetland et al. 2002; Hetland et al. 2003; Heuser & Norris 1946; Jones & Taylor 2001; Scott et al. 1954; Svihus et al. 1997), it is logical to assume that this will increase the digestibility and thus performance of the bird. Bale-Therik et al. (2012) concluded that grit had a positive effect on digestive performance on local chickens, and further studies should "*focus on the effect of grit on gizzard and nutrition metabolism on chicken (layer and broiler).*" Fritz (1937) found a slightly improved digestibility of coarse feed when poultry had acces to granite grit than no access to grit, which might indicate a positive interaction between digestibility and grit. Waugh et al. (2006) also concluded a positive effect of grit, and stated that grit supplementation for young ostriches should be recommended with respect to growth rate, feed efficiency and feed intake, this is also supported by Aganga et al. (2003).

When offering grit to birds, the shape of the stones should be round and not have any sharp edges to prevent potential damage to the gastrointestinal tract. For young ostriches Aganga et al. (2003) suggest round stones, made of insoluble materials such as quartz. Size of grit are also important; As mentioned, McIntosh et al. (1962) found better ME results with grower-sized rather than hen-sized grit. This might be because the relative gizzard size needs an adapted grit size to have an optimal function. Evans et al. (2005) offered zeolite either as powder or as grit, and found a significant effect of the grit shaped zeolite as it gave a better egg production, egg mass, feed conversion and AME, which supports that the shape of the grit affects its function. Moore (1998) concluded that smooth grit in form of glass beads did not have a good effect in the breakdown of grass in an artificial gizzard, and that actually the absence of the glass beads contributed to a better breakdown of grass. The author also found results that the smaller grit, compared to larger grit, had a better effect on breaking down the grass due to the relatively larger surface area.

As mentioned, particles have to be ground down to a specific size before entering the small intestines. Results from Ferrando et al. (1987) claimed that particles between 0.5-1.5 mm, in broilers, are small enough to be passed through the pylorus and further into the duodenum. Newer results from both Amerah et al. (2008) and Hetland et al. (2002) actually show that the particles leaving the gizzard in broilers are even smaller, and that a generally high proportion of the particles are below 0.1 mm. A positive interaction between grit and duodenal particle size were discovered by Hetland et al. (2003), as grit decreased the mean duodenal particle size. Moore (1999) found that the particles leaving the gizzard of goose were <0.5 mm long and 0.25 mm wide. Generally, the smaller the particles are when entering the intestines, the larger surface area the digestive enzymes have ability to work on and thus the potential for digestion is higher. Thereby, it is desirable that the particles entering the intestines are as small as possible to enhance this.

Overall, literature show various effects of grit for poultry, and there is no strong and exact conclusion of the usage of grit.

3 Material and methods

An experimental trial was conducted at the Animal Production Experimental Centre, at the Norwegian University of Life Sciences (NMBU), Ås, Norway, between the November 12th and December 4th 2015. The experiment was a part of a comprehensive study, where the effect of different types of grit and the interaction between whole wheat and grit were examined.

3.1 Treatments

There were four treatments; control, zeolite grit, granite grit and marble grit.

3.1.1 Zeolite

The zeolite grit, with a 1-2.5 mm dimension, were ordered from ZEOCEM, a.s. The chemical composition of the zeolite (Table 3.1-1) were given by ZEOCEM (2016) on a datasheet dated 11.01.2016 by Ekologické laboratóriá Spišská Nová Ves, Division of Laboratory Service, and only main elements were given.

Table 3.1-1 Average values for chemical composition of zeolite (ZEOCEM 2016).

Chemical Formula	Chemical name	Content (%)
SiO_2	Silicon dioxide	68.5
Al_2O_3	Aluminium oxide	12.8
TiO2	Titanium dioxide	0.17
Fe_2O_3	Iron(III)oxide	1.5
CaO	Calcium oxide	3.3
MgO	Magnesium oxide	1.1
MnO	Manganosite	0.03
P_2O_5	Phosphorus pentoxide	<0.05
Na_2O	Sodium oxide	1.4
K_2O	Potassium oxide	2.9
Ba	Barium	0.06
Sr	Strontium	0.02

3.1.2 Granite

The granite grit, with a 2.0-3.5 mm dimension, was ordered from Sibelco Nordic AB, a supplier of industrial minerals. The grit was produced by Woldstad Sandforretning in Norway. Chemical composition of the granite grit is presented in Table 3.1-2. As this grit contains a high amount of SiO_2 it is also possible to call it quartz.

Table 3.1-2 Average values for the chemical composition of the granite grit (Sibelco Nordic Sibelco n.d.).

Chemical Formula	Chemical name	Content (%)
SiO_2	Silicon dioxide	79.5
Al_2O_3	Aluminum oxide	9.5
K_2O	Potassium oxide	3.6
Na_2O	Sodium oxide	2.6
Fe_2O_3	Iron (III) oxide	2.0
CaO	Calcium oxide	1.7
MgO	Magnesium oxide	0.67
TiO_2	Titanium dioxide	0.28

3.1.3 Marble

The marble grit, with a dimension of 0.5-2.0 mm, also known as calcium carbonate, was produced by Visnes Kalk AS in Lyngstad of Norway. The chemical composition of the marble grit is presented in Table 3.1-3.

Table 3.1-3 Average values for the chemical composition of marble gritstone (Visnes Kalk AS 2007).

Chemical formula	Chemical name	Content (%)
$CaCO_3$	Calcium carbonate	98
$MgCO_3$	Magnesium carbonate	1
Fe_2O_3	Iron(III)oxide	0.1
SiO_2	Silica (quartz)	0.6

3.2 Experiment at the Ås Gård

The experiment at the research facility at Ås Gård was performed in collaboration with five other master students, Aorihan, Kari Borg, Biemujiafu Fuerjiafu, Huan Liu, and Sodbilig Wuryanghai. Hence, the material and method have been written in co-operation.

3.2.1 Animal housing

252 day-old male broiler chickens (Ross 308) were randomly placed into four equal sized pens (72cm x145cm). A thick layer of wood shavings covered the floors. The birds had access to both feed and water *ad libitum*. Room temperature the first week was approximately 28 ℃. Extra heating was provided by heat lamps over the pens the first 5 days to ensure that the chickens were in their thermal neutral zone (approximately 30 °C). Room temperature was reduced down to 22 °C over the three following weeks. On day 5 birds were moved from the pens to quail cages (depth 35 cm x width 50 cm x height 20 cm). 4 birds from one pen where randomly selected and placed in one quail cage, this was repeated 12 times for each pen, giving in total 4 birds x 12 replicates x 4 treatments = 192 birds divided on the 48 quail cages. Birds below 130 grams were excluded from the experiment. The extra birds were left in their pens, and did not participate further in the experiment. The birds were exposed to continuous lightning due to no possibility for complete darkness at the facility. The quail cages were equipped with both a feeder and a water container, and trays under to collect excreta. The quail cages were organized in two sections. Each side of each section contained three rows with four cages. The treatments were distributed among rows, and the patterns changed for each side of the sections.

3.2.2 Experimental plan

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

Diet and grit inclusion

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

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

Bird weight were registered on day 5, 11, 13, 18, 21 and 22. The feed consumption was measured at the same time, starting from day 5-11. Quantitative sampling of excreta was conducted from 5-11, 11-13, 13-18 and 18-21 days of age. These samples were frozen for further analysis.

<u>Dissection and starvation</u>

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

At day 20 feed was taken away at 21:00 and the birds were starved until 07:00 on day 21, where feed was again provided. On day 21, 1 of 2 bird was removed from each quail cage, marked with its cage number, and placed in a pen corresponding to its treatment with access to water and feed. The excreta trays were removed and cleaned. The remaining birds in the quail cages got access to feed for 5 hours, and the excreta trays were placed back after two hours of access to feed to collect excreta. The trays where left to collect excreta produced during the following 3 hours. After 5 hours, the birds was killed with a cranial blow followed by cervical dislocation and dissected. After dissection of all 48 birds, the birds in the pens were placed back into their respective quail cage and given access to feed and water.

At day 21 feed was taken away at 21:00 and the birds were starved until 07:00 on day 22. On day 22, the birds were given access to feed for only 30 minutes. Two birds from each treatment were killed with a cranial blow followed by a cervical dislocation, 60, 90, 120, 150, 180 and 210 minutes after commencement of feeding.

3.2.3 Laboratory work

All the samples were first thawed then homogenized, respectively.

Dry matter:

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

A representative sample was taken out, wet weight registered, and then dried in an oven at 105 ± 2°C over the night. The sample was placed in a desiccator until cooled and the dry weight was measured. Tare weight of crucible were subtracted from the gross weight of the sample to calculate net weight of wet/dry sample (equation 1).

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

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

AME

Apparent metabolizable energy (AME) of faeces from day 13-18 and 18-21 were performed by lab technician Frank Sundby according to NMBU’s procedure; a representative sample of the homogenized faeces were dried overnight (105 ± 2°C) and put in a bomb calorimeter (PARR 6400 Bomb Calorimeter) and values were calculated for each sample. For the grit treatments, grit was included in the sample as it was not possible to remove before analyzing.

Gizzard pH:

Before the dry matter was determined in the gizzard content, pH was measured by pH meter (VWR pH100)

Separation of grit from in the gizzard and faeces

Due to a relatively small amount of gizzard content, the whole sample including grit had to be used for dry matter determination. Thus, the dried gizzard content had to be dissolved in water before separating grit and feed in the gizzard content. The method to separate the particles 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 (feed particles) floated up and were washed out, while the high density particles (grit) were left in the bottom of the bowl. The grit was then dried in room temperature overnight and weighed the following day, and saved for further analysis.

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

Wet sieving procedure

Wet sieving of faeces was done to determine the particle distribution on dry matter basis. Faeces collected from days 11-13, 13-18 and 18-21 were first homogenized and analyzed 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 (described in the next paragraph) 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) on setting 3, before it was 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. Sieve sizes (all in mm) were 1.4, 0.8, 0.5 and 0.2, and water pressure was at maximum. Sieving time

was set to 2 minute with water, and 1 minute without water to shake off excess water. Each sieve was then weighed with sample and weight was registered.

From 4 replicas per treatment for all sample sets, a sample of approximately 2.5 grams was 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 described above and weighed. The average of 11 registrations were used when subtracting the tare weigh from the gross registration of the wet sample. The content left in the sieves was washed out in a bowl and rinsed for grit as described in "Separation of grit from in the gizzard and faeces", and the grit was collected and saved for further analysis.

Particle distribution of grit

Particle distribution of the initial grit (as fed), grit in faeces and gizzard content were determined.

Samples from the initial grit were dry sieved to find the actual particle size distribution of grit given to the birds. Sieve size (all in mm) were 1.4, 0.8, 0.5 and 0.2, and additionally the collector bin (<0.2mm) were used. The tare of each sieve, including the collector bin, was first registered before 100 grams of the initial grit were dry sieved for 1 minute on amplitude 1.00 mm/g on the Retsch sieve shaker (AS 200 Control), each sieve was then weighed and registered again before emptying the content of the sieves. All steps where repeated between each sample. Each type of grit were sieved 4 times to get an average particle distribution.

Same procedure was conducted for grit amount that were found in the faeces and gizzard. For the grit in the gizzard, only zeolite and granite were recovered and since the samples of grit from the gizzard content was very small, the samples were pooled together from 12 replicas to 3 replicas so that the total sample were approximately evenly distributed within the treatments.

The percentage particle distribution were calculated with equation 2, whereas “n” represent sieve size.

$$\% \text{ of particle of } n = \frac{\text{weight of } n_{with\ sample} \text{ (g)- weight of } n_{empty} \text{ (g)}}{\text{weight of total sample (g)}} \times 100 \qquad (2)$$

Due to human error, the particle distribution of initial grit were measured of the remaining grit left the bag after the end of the experiment. However, the particle distribution were assumed to be equal in the bag. A previous sieving had been done before the start of the experiment to get a quick picture of the actual particle size, but with only one replica of 500 grams and a different set of sieve sizes (3.55, 3.5, 2.8, 2.5, 2.0, 1.6, 0.8 and 0.5 mm).

3.2.4 Statistical analysis

Professor Birger Svihus, using a SAS software, performed statistical analysis on all data except the particle distribution of grit in gizzard which were performed in R statistical software by myself. Statistical differences that are significantly different ($P<0.05$) are marked with different superscript. No superscript means no overall significant difference.

Each treatment had 12 replicates, and thus the numbers presented in the results are the average value for these replicates with two exceptions; On day 11 one bird (from quail cage 45) from the marble treatment was killed due to leg problems, and on day 20 one bird (from quail cage 48) from the granite treatment was also killed due to health problems. Weight of the dead chicken and feed for the quail cage were registered on the given days. This were accounted for when calculating the data shown in the results.

4 Results

As this master thesis focus on the granite treatment, results comparing the control and the granite treatment are discussed. The only comparison of granite and the other treatments are discussed in chapter 4.3 - Grit passage and distribution in the digestive tract as it is not possible to compare with the control, because it did not receive any grit.

4.1 Initial grit particle distribution

Initial particle distribution (%) of the grit as fed is presented in Figure 4.1-1. Distribution of granite particles was 95% above 1.4 mm, 4% for particles between 0.8 and 1.4 mm and 1% for particles between 0.5 and 0.8 mm.

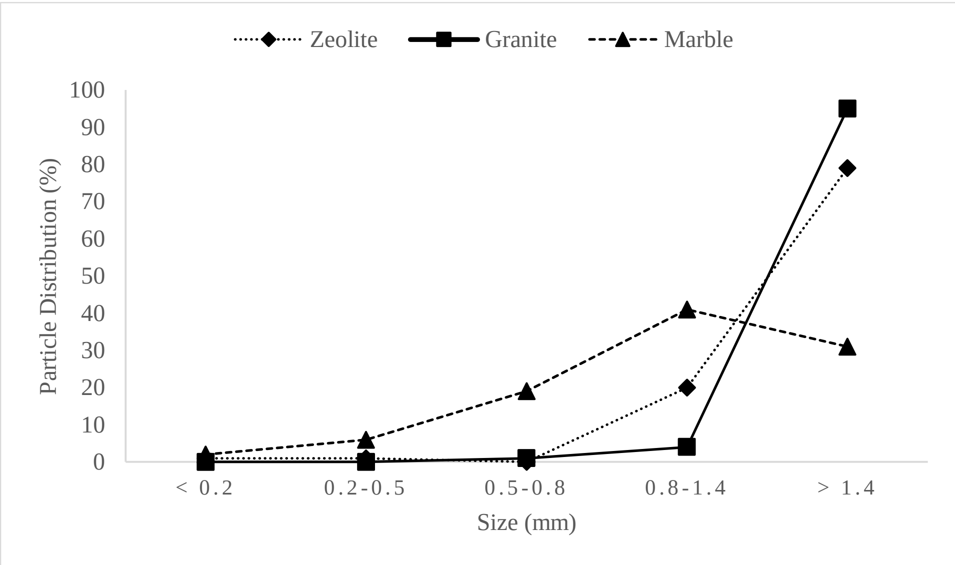

Figure 4.1-1 Initial particle distribution (%) of zeolite, granite and marble grit as fed.

4.2 Bird performance

There were no significant differences for the granite treatment compared to the control on performance (Table 4.2-1). There were also no significant difference on the AME for the granite compared to the control (Table 4.2-1), even though the diet was changed from grower diet on day 18, to a more digestive challenging diet consisting of 15% whole wheat and 85% starter diet.

Table 4.2-1 Performance data for the birds; weight gain (g), feed intake (g), feed:gain and apparent metabolizable energy (MJ/kg). Figures in columns not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

		Control	**Zeolite**	**Granite**	**Marble**	**Sq. MSE***	**Significance**
Weight gain (g)	**5-11 days**	225a	219a	225a	203b	15.8	0.0044
	11-13 days	139	145	145	138	10.7	0.2535
	13-18 days	384ab	406a	384ab	361b	24.7	0.0009
	18-21 days	135	152	143	124	28.2	0.0956
	5-21 days	882a	921a	893a	825b	47.6	0.0001
	11-21 days	657bc	702a	672ab	622c	43.3	0.0006
	5-18 days	748a	769a	754a	702b	35.6	0.0003
Feed intake (g)	**5-11 days**	275a	269a	271a	252b	10.2	<.0001
	11-13 days	161ab	170a	165ab	157b	9.9	0.0174
	13-18 days	495a	516a	496a	467b	26.9	0.0009
	18-21 days	270ab	281a	283a	246b	29.6	0.0137
	5-21 days	1201a	1235a	1215a	1121b	53.2	<.0001
	11-21 days	926a	966a	944a	870b	49.4	0.0002
	5-18 days	931a	955a	932a	876b	37.0	<.0001
Feed:gain	**5-11 days**	1.23	1.23	1.21	1.24	0.053	0.4888
	11-13 days	1.16	1.18	1.14	1.14	0.057	0.3641
	13-18 days	1.29	1.27	1.29	1.30	0.038	0.4497
	18-21 days	2.05	1.88	2.07	2.10	0.409	0.5578
	5-21 days	1.36	1.34	1.36	1.36	0.040	0.6490
	11-21 days	1.41	1.38	1.41	1.40	0.049	0.4023
	5-18 days	1.25	1.24	1.24	1.25	0.028	0.7811
AME (MJ/kg)	**13-18 days**	13.5	13.6	13.7	13.6	0.40	0.7760
	18-21 days	14.0	14.1	14.2	13.5	1.04	0.3500

* Sq. MSE: Square of Mean Square Errors. The variance of the means values, also known as the standard deviation.

4.3 Grit passage and distribution in the digestive tract

Grit results are presented in Table 4.3-1. Birds consumed 37 grams of granite (97% of given amount). Quantitative grit passage was only recorded until day 18.

On day 13, 3.13 grams of granite were recovered in the gizzard. On day 18, the amount had decreased to 1.64 grams. Between day 5 and 18, 11% of the eaten granite amount had disappeared and could not be accounted for in either faeces or gizzard. On day 11, 45% of granite eaten were recovered in the faeces and had thus passed though from day 5 to 11. Further 12% and 10% of eaten granite were recovered in faeces between day 11-13, and day 13-18 respectively.

Both granite and zeolite had significantly higher amount grit consumed compared to marble ($P<0.0001$). Granite also had a significantly higher amount grit retained in the gizzard than the other treatments on both day 13 ($P<0.0001$) and day 18 ($P<0.0002$). Amount granite grit disappeared is significantly lower compared to the other treatments ($P<0.0001$). The passage of granite from day 5 to 11 were significantly higher than marble ($P<0.0001$), but of no significance compared to zeolite. The passage of granite from 11 to 13 days were significantly lower compared to the other treatments ($P<0.0065$). The passage of granite from 13 to 18 days were significantly higher compared to the both other treatments ($P<0.0001$).

Table 4.3-1 Values for grit consumption (g), grit content in gizzard (g), disappearance[a] (%) and passage[b] (%) at different ages. Figures in columns not sharing common superscript are significantly different ($P<0.05$). No superscript means no overall significant difference.

	Control	Zeolite	Granite	Marble	Sq. MSE[*]	Significance
Grit eaten 5 to 11 days, g	-	37a	37a	23b	4.5	<.0001
Grit in gizzard 13 days, g	-	0.86b	3.13a	0c	0.575	<.0001
Grit in gizzard 18 days, g	-	0.09b	1.64a	0b	0.937	0.0002
Grit disappearance[a] 5 to 18 days, %	-	34b	11c	54a	10.6	<.0001
Grit passage[b] 5 to 11 days, %	-	39a	45a	26b	8.5	<.0001
Grit passage[b] 11 to 13 days, %	-	18a	12b	20a	6.2	0.0065
Grit passage[b] 13 to 18 days, %	-	7b	10a	0c	4.2	<.0001

[a)] Percentage of eaten amount not accounted for in faeces or gizzard.
[b)] Percentage of eaten grit recovered in faeces.
[*] Sq. MSE: Square of Mean Square Errors. The variance of the means values, also known as the standard deviation.

As only zeolite and granite grit were recovered in the gizzard, Figure 4.3-1 shows the particle distribution of these grits in gizzard at days 13, 18, 21 and 22. At day 13, granite had a significant lower proportion of particles between 0.8 and 1.4 mm (P<0.0212), and a significant higher proportion of particles above 1.4 mm (P<0.0286). The same significance pattern repeated itself at day 21, as granite was significant lower than zeolite for particles between 0.8 and 1.4 mm (P<0.0075) and significant higher than zeolite for particles above 1.4 mm (P<0.0078).

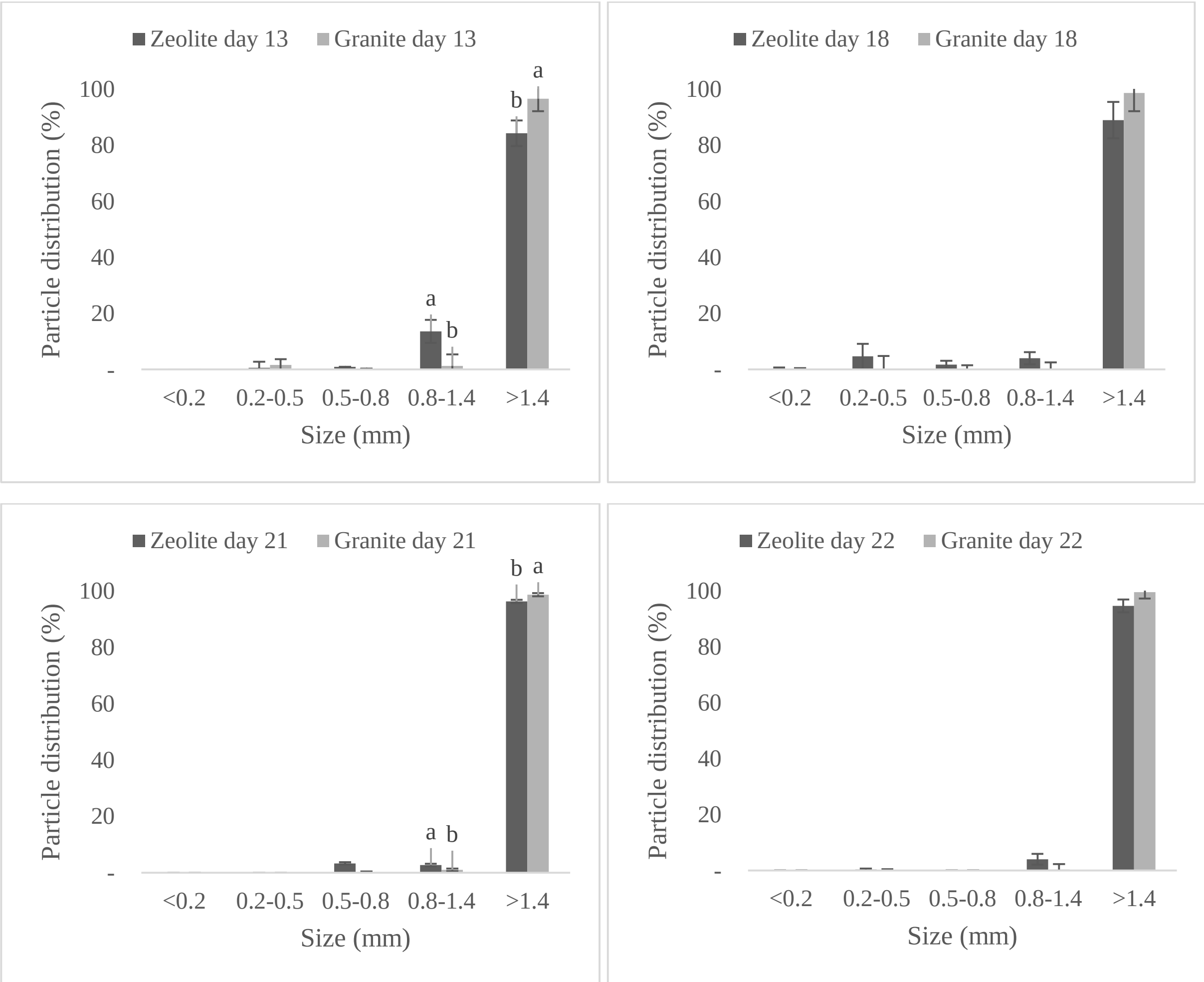

Figure 4.3-1 Particle distribution (%) of zeolite and granite grit recovered in the gizzard on day 13, 18, 21 and 22. Bars not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

The particle distribution of grit recovered in faeces collected on different days are presents in Figure 4.3-2, Figure 4.3-3 and Figure 4.3-4. As particles of non-grit origin from the control was not corrected for, the figures shows the distribution included disturbance from the feed. The particle distribution from the control is presented in Table 4.3-2. The statistical analysis were run with the data from the control, therefore different superscript shows statistical differences with the control. This section will discussed further in chapter 5.3.

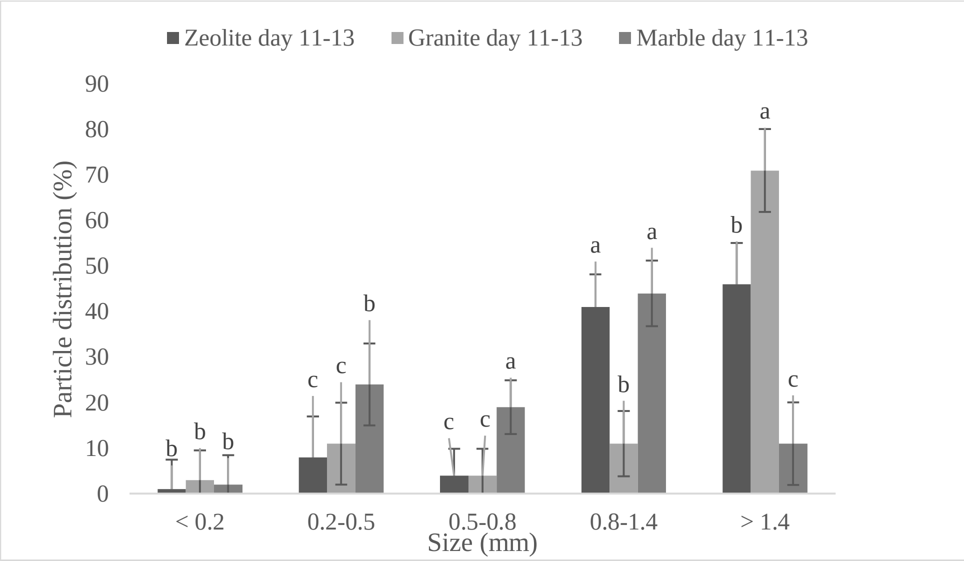

Figure 4.3-2 Particle distribution (%) of grit recovered in faeces, included disturbance from the feed, collected between day 11-13. Bars not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

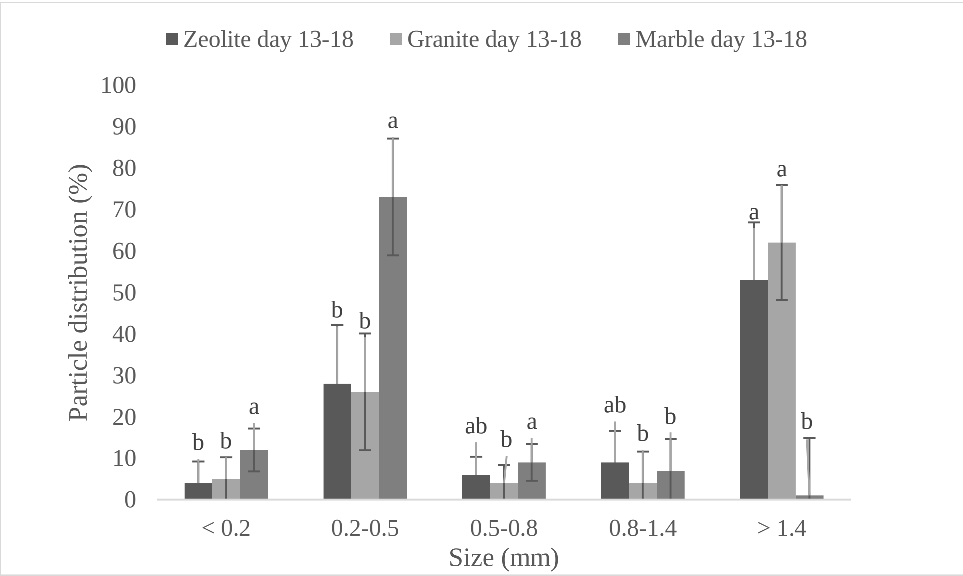

Figure 4.3-3 Particle distribution (%) of grit recovered in faeces, included disturbance from the feed, collected between day 13-18. Bars not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

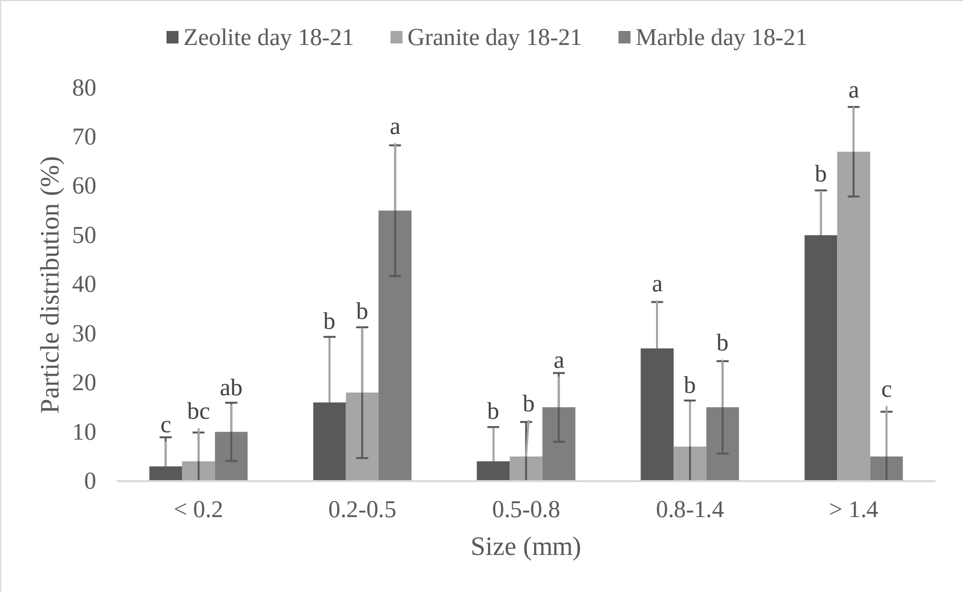

Figure 4.3-4 Particle distribution (%) of grit recovered in faeces, included disturbance from the feed, collected between day 18-21 Bars not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

Table 4.3-2 Particle distribution (%) of particles of non-grit origin recovered in faeces from the control collected between day 11-13, 13-18 and 18-21. Figures in columns not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

	< 0.2	**0.2-0.5**	**0.5-0.8**	**0.8-1.4**	**> 1.4**
Control day 11-13	12a	69a	11b	7b	2d
Control day 13-18	12a	61a	10a	15a	4b
Control day 18-21	14a	62a	15a	6b	3c

4.4 Gizzard characteristics

No data comparing the control and the granite group of the gizzard characteristics were of any significance, except for the empty gizzard weight of 13 days which were significantly higher than the control (P<0.0051) (Table 4.4-1).

Table 4.4-1 Gizzard performance results; content wet weight (g), empty gizzard weight (%), relative gizzard weight (%), gizzard pH, and bird weight (g). Figures in columns not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

		Control	Zeolite	Granite	Marble	Sq. MSE*	Significance
Gizzard content (g)	**21 days**	8.6ab	9.6ab	10.2a	7.2b	2.51	0.0296
	18 days	8.1	7.1	8.1	8.1	5.11	0.9559
	13 days	6.6ab	6.1b	8.5a	5.7b	1.96	0.0076
Empty gizzard (g)	**21 days**	16.5	16.8	16.7	15.3	1.90	0.1853
	18 days	14.0	14.0	13.8	13.4	2.11	0.8723
	13 days	10.5b	11.0ab	11.7a	10.3b	0.97	0.0051
Relative gizzard content (%)**	**21 days**	0.82	0.86	0.94	0.73	0.212	0.1156
	18 days	0.89	0.79	0.91	0.97	0.559	0.8879
	13 days	1.34ab	1.24b	1.63a	1.14b	0.394	0.0230
Relative gizzard weight (%)***	**21 days**	1.59	1.52	1.56	1.54	0.172	0.8105
	18 days	1.56	1.58	1.55	1.61	0.256	0.9313
	13 days	2.14	2.21	2.26	2.05	0.226	0.1356
Gizzard pH	**21 days**	3.0	2.8	2.9	2.8	0.48	0.6643
	18 days	3.3	3.6	3.6	3.1	0.80	0.3888
	13 days	3.1	3.5	3.4	3.5	0.59	0.3280
Bird weight (g)	**21 days**	1043	1107	1070	996	104.7	0.0823
	18 days	898	898	894	838	73.0	0.1315
	13 days	495	497	518	504	31.5	0.2953

* Sq. MSE: Square of Mean Square Errors. The variance of the means values, also know as the standard deviation.
** Gizzard content expressed as percentage of body weight.
*** The empty gizzard weight expressed as a percentage of the body weight.

4.5 Particle distribution of faeces

The particle distribution (%) of faeces is presented in Figure 4.5-1, Figure 4.5-2 and Figure 4.5-3. For granite compared to the control, there were a significant decrease in particle distribution below 0.2 mm (P<0.0002) and a significant increase in particle distribution between 0.8 mm and 1.4 mm (P<0.0001) for faeces collected between day 11 and 13. There were no significant differences between granite and the control between dag 13 and 18. There were a significant increase in particle distribution between 0.8 mm and 1.4 mm (P<0.0029) for faeces collected between day 18 and 21. Unfortunately, only the data from day 11-13 and

13-18 were not corrected for grit when statistical analyzed, only the data from day 18-21 is corrected for grit.

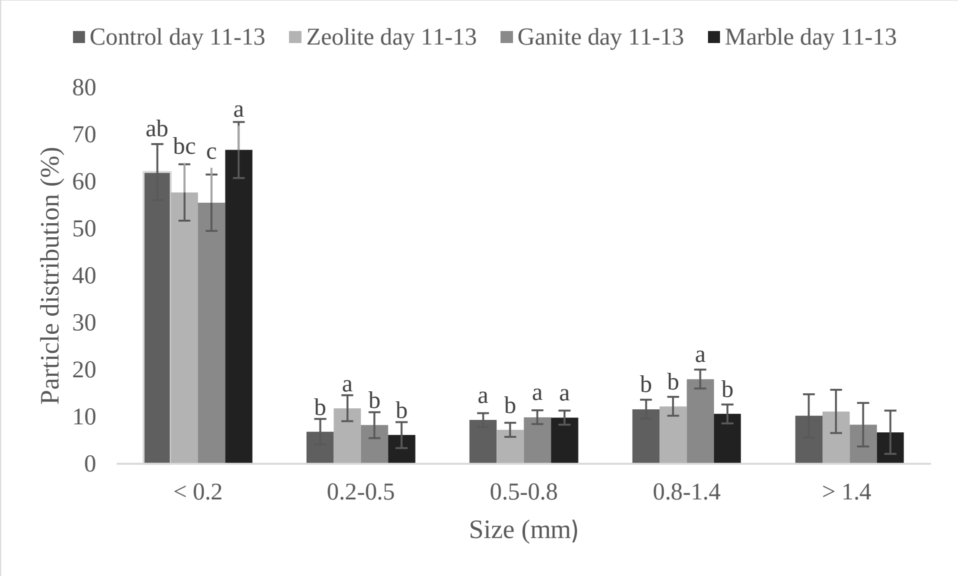

Figure 4.5-1. Particle distribution (%) of faeces, not corrected for grit, collected from day 11 to 13. Bars not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

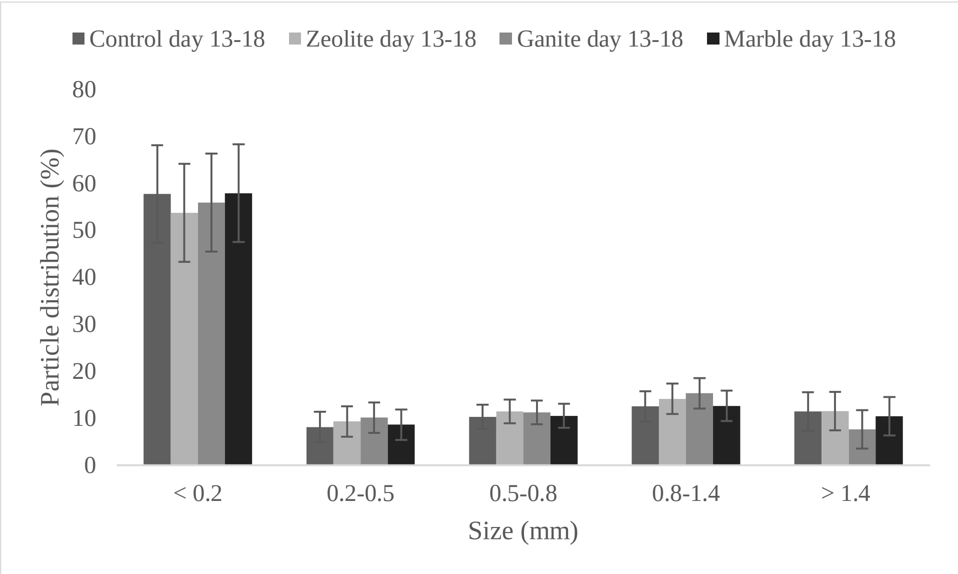

Figure 4.5-2. Particle distribution (%) of faeces, not corrected for grit, collected from day 13 to 18. Bars not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

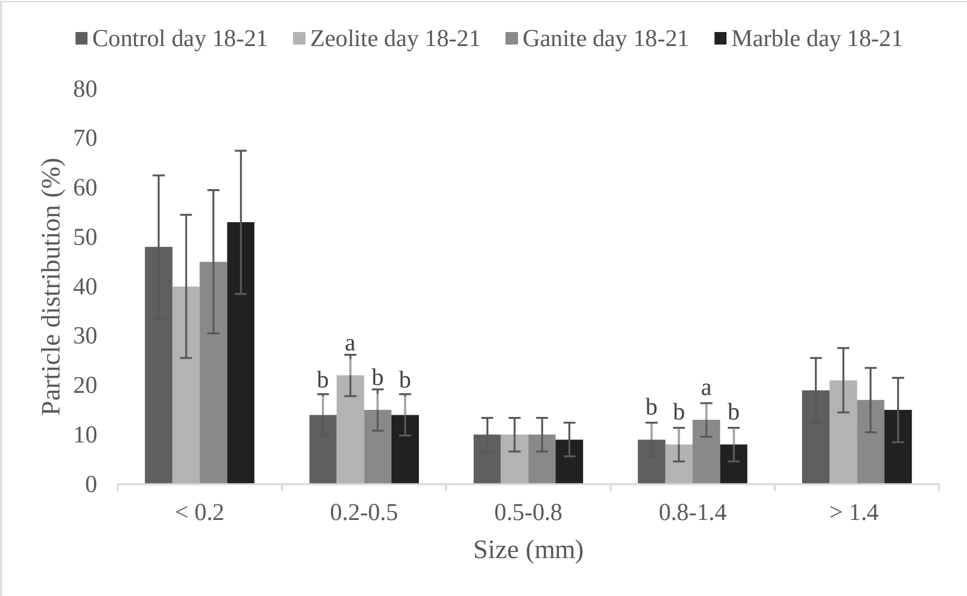

Figure 4.5-3. Particle distribution (%) of faeces, corrected for grit, collected from day 18 to 21. Bars not sharing common superscript are significantly different (P<0.05). No superscript means no overall significant difference.

4.6 Effect of granite content on empty gizzard weight

There was no correlation between empty gizzard weight (g) and granite content (g) in gizzard, see Figure 4.6-1. There was also no correlation between relative gizzard weight (%) and granite content (g) in gizzard (figures not shown). R^2-values for relative gizzard weight and granite content were; $R^2_{13} = 0.0998$, $R^2_{18} = 0.1591$, $R^2_{21} = 0.0525$ and $R^2_{21} = 0.0813$.

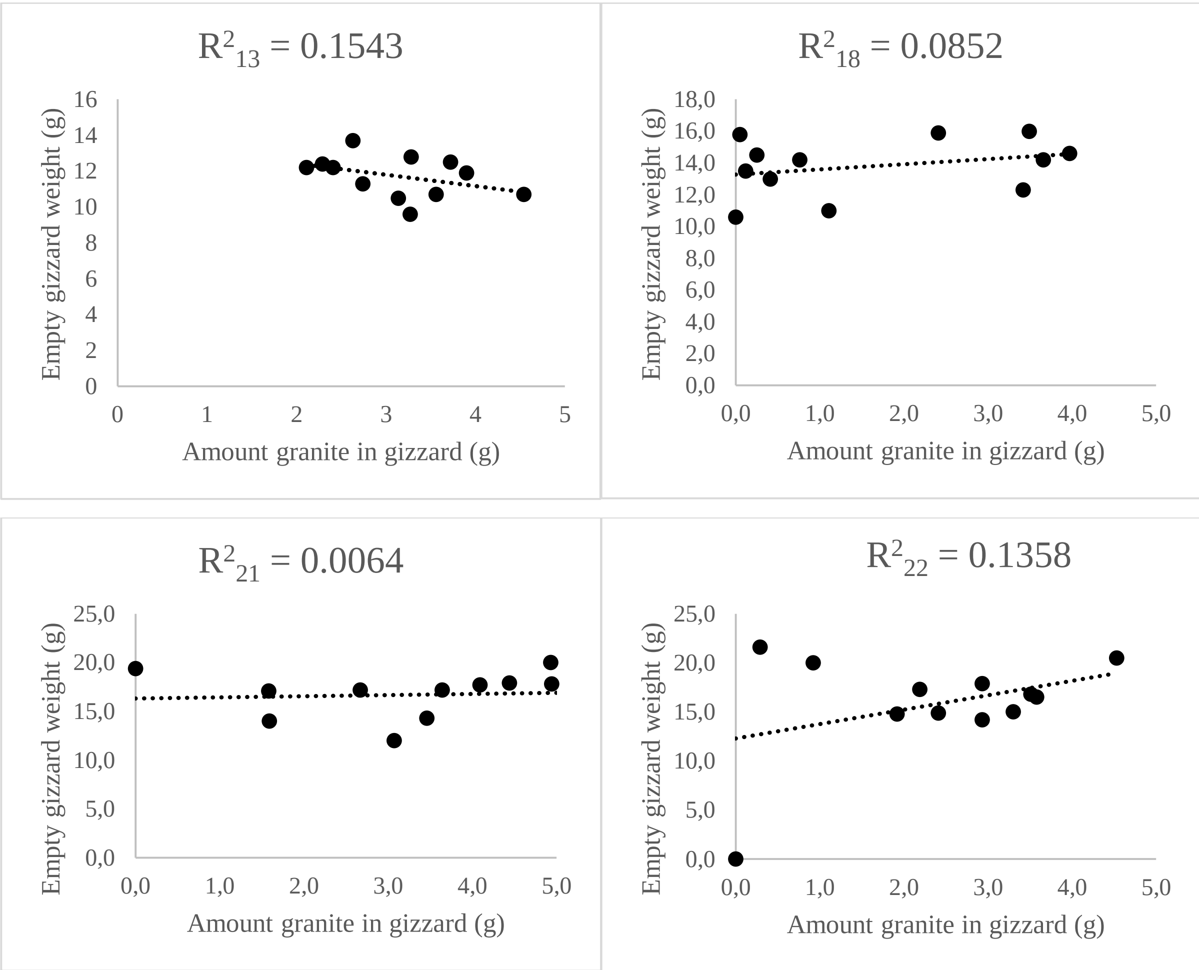

Figure 4.6-1. Correlation between empty gizzard weight (g) and amount granite (g) on different ages (day 13, 18, 21 and 22).

5 Discussion

The actual need for grit as a feed utilizing enforcer for poultry is in general questioning, as literature shows different results. Todays' feed technology is very advanced and can utilize the nutrients in the raw materials to a great degree. In combination with heat, moisture and enzyme addition, it is possible to increase the availability and digestibility of several nutrients. Other feed processing parameters such as grinding, pelleting, extrusion and expansion affects factors like feed intake, nutrient availability and nutrient utilization. It is possible that the digestive tract of todays' poultry have grown adapted to this and thereby the need for grit as a grinding agent to utilize nutrients is lost. Garipoglu et al. (2006) stated that due highly digestible diets, the digestive tract of birds have grown lazy, and thereby the bird might is dependent on feed technology to maximum utilize the nutrients in the feed.

5.1 Initial grit particle distribution

As the majority of the initial granite particle distribution was above 1.4 mm as fed (Figure 4.1-1), it was assumed granite grit particle distribution was consistent with the manufactures' information about a dimension between 2.0-3.5 mm. This sieving was performed after the experiment had ended, and might not have been performed on a representative sample, as the granite that was left in the grit bag was under 1 kg.

An additional dry sieving of the granite as fed (data not shown) with sieve sizes (all in mm) of 3.55, 3.5, 2.8, 2.5, 2.0, 1.6, 0.8, 0.5 and collector bin showed a mainly distribution of 48% of 2.0-3.55 mm and 39% of 1.6-2.0 mm. This was only done on one replica for a sample of 500 grams granite. This additionally sieving was done before the experiment started, and thus might be more representative than the sieving conducted after the experiment ended.

Overall, it was assumed that the initial granite particle distribution was consistent with the manufactures' information of a dimension of 2.0-3.5 mm.

5.2 Bird performance

As there was no significant effect of granite, these results supports findings from Bethke and Kennard (1926) and Garipoglu et al. (2006), both authors also used granite grit and found no significant performance effects of granite.

The birds were given grit on day 5, 7 and 9 and had access to it until day 11 before it was removed. In this time, 97% of all grit was eaten, and thus the potential for an effect of granite, due to the high intake, were highly possible. The experiment was also conducted over three weeks, and thus the time should have been enough to see an effect of granite. However, either of these factors contributed to an effect of granite. Balloun and Phillips (1956) reported an increased weight gain and reduced feed:gain after six weeks when young chickens were fed *ad libitum* grey granite, red quartz and a combination of both grit types, which implies the positive potential grit has on chickens. As mentioned Balloun and Phillips (1956) offered grit *ad libitum*, and on average over a three week period, the birds consumed 62 grams grey granite, 46 grams red quartz and when fed the combination they consumed 29 grams of grey granite and 26 grans red quartz. This is a higher amount than the 37 grams eaten granite in the present study. Another difference is that in the present study a commercial pellet diet was used, in contrary to mash rations used by Balloun and Phillips (1956), and period for their experiment were from three to six weeks of age while the present study were from one day of age and to three weeks of age. Based on this, amount of grit and age when introduced to grit may affect the results. As of today, if grit shall have an effect of broiler chicken performance, it is important that this effect will occur early in the birds' life as modern broilers live approximately 30-32 days.

It is a possibility, that the combination of a well developed feed and broiler breed, does not require granite to maximize the utilization of feed, and thus growth, feed intake and feed:gain does not improve by grit supplementation. Even when the diet was changed to a diet with structural components, whole wheat, granite did not have a significant effect on performance parameters. A significant increase of AME for granite from day 18-21 would indicate that granite increased the digestibility potential of whole wheat, thus improving the utilization of nutrients in whole wheat. This was not the case in the present study, and thus granite did not have a positive, nor a negative, effect on performance and energy utilization. These findings are contrary to McIntosh et al. (1962), who found an increase in ME when offered grower-sized granite rather than hen-sized grit. McIntosh et al. (1962) also stated that size of the grit might influence its effect on the bird. As the birds consumed almost 100% of offered granite, it is therefore assumed that the size of the granite was in a preferred range.

The chemical composition of the grit might have an influence on the performance as well. Bale-Therik et al. (2012) offered different levels of a type of grit mainly containing soluble calcium (approximately 80%) and concluded that this grit gave a positive effect on performance parameters such as average daily gain, feed intake, average daily body weight gain and feed conversion ratio. Whereas other studies have found severe negative effects of *ad libitum* access to limestone on culling rate and egg development (Blount (1948) in Branion & Heuser, *Grit for poultry: Bibliography and abstracts*; Fuller 1958). The present study did not have any noticeable mortality except for two birds, these cullings' were concluded as random and that no treatment effected mortality.

5.3 Grit passage and distribution in the digestive tract

As the birds had *ad libitum* access to feed, granite was most likely not utilized as a satiety factor due to lack of feed. As the birds consumed 97% of given amount of granite, it appeared that the birds liked granite and voluntary ingested it to a high extent when it was offered.

Since the birds did not get access to more granite after day 11 and until day 18, and the amount in the gizzard decreased by 48% from day 13-18 (3.13 gram granite to 1.64 gram granite, see Table 4.3-1), this might indicate that the granite is ground down and passed through the digestive tract or ground down to a irretrievable size. Although not documented in this thesis due to human errors, a subjective observation was that the granite in the gizzard became smoother and less sharp over the different ages (13, 18, 21 and 22). Which indicated that the grit is "worn" and thereby decreased in size.

As the initial granite as fed, had a distribution of 95% above 1.4 mm, it is logic that the majority of the particles recovered in the gizzard should be above 1.4 mm. However, as the birds did not get new access of grit after day 11 and the amount of granite in the gizzard decreased by 48% from day 13-18, and the distribution of granite particles in the gizzard was rather consistent with a general distribution of 95% of particles above 1.4 mm for both days (even day 21 and 22) (Figure 4.3-1). Thus, this might indicate that, as granite particles above

1.4 mm are retained in the gizzard, and as they are ground below 1.4 mm, they are either passed though further in the digestive tract or absorbed. This also supports Ferrando et al. (1987) theory about particles between 0.5-1.5 mm passes from the gizzard and into the digestive tract.

Only 11% of eaten granite could not be accounted for in either gizzard or excreta, this is approximately 4 grams. Grit found right next to the feed container on the collecting trays and were registered as “spill grit” and accounted for when calculating the eaten amount. The disappeared amount might be due to human error such as when looking for “spill grit” some particles were not found, incorrect measurements of given stone or even the chickens might have eaten so enthusiastic that some grit jumped out from the feeder and did not end up on the collecting trays. It was observed that some pellets could jump out of the feeder when the birds ate very enthusiastically. Even though the commercial diets all were balanced regarding mineral needs, it is a possibility that the birds utilized granite as a mineral source and thus this amount could not be accounted for as it was absorbed.

On average, approximately 25 grams eaten granite were recovered in the faeces until day 18 (Table 4.3-1), which is approximately ⅔, while Vance (1971) only recovered ¼ in faeces, which shows that insoluble grit, compared to soluble grit (Korschgen et al. 1965), is more easily recoverable to some extent in bird faeces.

Grit retained in gizzard on day 13 (Figure 4.3-1) was significantly higher for granite compared to zeolite, this can be explained by a significantly lower passage rate for granite between day 11 to 13 (Table 4.3-1). The results of grit retained in gizzard at day 18 (Table 4.3-1) showed a significantly higher retention of grit in gizzard for granite compared to zeolite, but the passage rate was significantly higher for granite than zeolite between day 13 and 18. Therefore, the disappearance percentage explains the differences between granite and zeolite better. Granite has a much lower significant disappearance percentage than zeolite, 11% versus 34%, which indicates that zeolite is to a much higher extent ground down to an irretrievable size or absorbed.

Results comparing granite and marble, shows an overall better grit performance for granite in comparison with marble (Table 4.3-1). Only 61% of the offered marble was eaten, and no marble was recovered in the gizzard. When offering *ad libitum* limestone grit, Jones and

Taylor (1999) recovered 3.4 grams limestone in the gizzard and thus it should be possible to recover marble in the gizzard. Only of 46% of eaten marble was recovered in the faeces, while 54% disappeared and could not be accounted for. Most likely, the marble was dissolved and absorbed, or ground down to an irretrievable size. Marble is the only grit which could be 100% accounted for, as the disappearance and passage rate ads up to 100%. For zeolite and granite, the sum in 98% and 78% respectively, thus estimating that after day 18 birds had 2% and 22% of zeolite and granite respectively in their digestive tract. This is approximately 0.74 grams zeolite and 8.14 grams granite. Since grit passage only was recorded until day 18, it is impossible to say where in the digestive tract the grit was. It can be postulated, based on the results, that granite would most likely be retained in the gizzard to a larger extent than zeolite.

Results of the particle distribution of grit recovered in faeces (Figure 4.3-2, Figure 4.3-3 and Figure 4.3-4) are of uncertainty, as they were not corrected by particles of non-grit origin found in faeces in the control and thereby consist of disturbance. When wet sieving the faeces from the control, small particles of non-grit origin were recovered and saved for particle distribution analysis. Unfortunately, the particles were not corrected for when processing the data, and thus represents disturbance in the statistical analysis. Looking at Table 4.3-2, it shows that the majority of the particles from the control are between 0.2 and 0.5 mm for all collection periods, making the results regarding these sizes very uncertain. As basically no particles from the control were above 1.4 mm (Table 4.3-2) for all collection periods, it can be assumed that the distribution for particles above 1.4 mm in Figure 4.3-2, Figure 4.3-3 and Figure 4.3-4 were only grit particles. Which shows that the majority of the grit particles from zeolite and granite are above 1.4 mm for all collections periods. The particle distribution of the grit were calculated from the grit recovered in 100 grams wet faeces, as described in chapter 3.2.3 – Wet sieving procedure. Generally, this sample were below 1 gram grit/100 grams wet faeces, and thus is not very representative. A much larger wet faeces sample should have been separated for grit, dried and then sieved to calculate the particle distribution of grit in faeces.

5.4 Gizzard characteristics

Even though the birds ate a high amount of granite, it did not have any effect on gizzard characteristics except for a significantly higher empty gizzard weight at day 13, but the relative gizzard weight at day 13 were not significant. The relative gizzard weight is a better measurement as it considers the birds' body weight, and thus the proportions between organ and bird size. Although it is noticeable that the empty gizzard weight for granite is significantly higher compared to the control at the maximum point for grit stimulation. At day 11 the birds had been offered grit on day 5, 7 and 9, therefore the birds had continuously been exposed to grit in their feed. This might have accelerated the gizzard growth until this age, and thus increased empty gizzard weight at day 13, before the growth of the gizzard stabilized. At a later age, there is no significant effect of granite on gizzard characteristics, this might be due less amount of granite in gizzard as discussed in chapter 5.3, or simply the possibility that granite does not have an overall effect on gizzard characteristics over time. Garipoglu et al. (2006) found an increase of gizzard weight when offering granite *ad libitum* and their birds consumed on average 2.7 grams/day between day 7-14 and 4.4 grams/day between day 15-21. While the birds in the present study were only given 9.5 grams granite/bird in total from day 5 to 9 and 1 gram granite/bird from day 18-20. There might be a possibility that if the birds in the present study had been offered more granite this would have had an effect on gizzard characteristics.

As mentioned in chapter 2.3, Spencer and Jenkins (1963) found an increase of gizzard size due to flint grit. The authors postulated that the increase was due to an increased gizzard activity and thus an increased muscle growth. Scott and Heuser (1957) found that grit would accelerate the development of the gizzard, but it would reach a maximum point regardless of grit or no grit treatment. Therefore, the effect of grit decreased over time, which is also to some extent seen in this study, as the only significant effect of granite was on day 13 on empty gizzard weight while at later ages there were no significant differences between the control and the granite on gizzard development. As both Spencer and Jenkins (1963) and Scott and Heuser (1957) experiments was conducted over 50 years ago, and poultry have generally short generation intervals, it might be possible that modern breeding have bred away this response to grit.

Several authors have found an effect of structural components, such as wood shavings, whole cereals and grains, and gizzard weight (Amerah et al. 2008; Hetland et al. 2002; Svihus et al. 1997). The chickens in the present study were given whole wheat from day 18, and there were no significant effects of granite x whole wheat on gizzard development. This might me due to a too small and short exposure to whole wheat. If whole wheat had been introduced at an earlier stage, it is possible that this could have had an effect on the gizzard.

5.5 Particle distribution of faeces

In general, for all collection periods, the majority of the faeces particles are below 0.2 mm for both control and granite. Which is supports Ferrando et al. (1987) regarding particle size of 0.5-1.5 mm to be suitable to pass though the gizzard and into the duodenum, and Hetland et al. (2002) and Amerah et al. (2008) that the majority of these particles are below 0.1 mm. Even though particles below 0.2 mm were not divided into smaller size in this experiment, the results show clearly that the particle distribution is favored for particles below 0.2 mm in faeces.

If granite had a clear positive effect on particle distribution, it is assumed that the smallest particle distribution would be significantly higher for the granite than the control. The results shows the opposite for particle distribution between day 11-13 (Figure 4.5-1) regarding the particles below 0.2 mm, while “large” particles between 0.8 and 1.4 mm are significantly higher for granite than the control at both day 11-13 (Figure 4.5-1) and day 18-21 (Figure 4.5-3). Although, this results were rather random since there is no pattern of the same significant effect on the different collections periods. If the general significance were identical for all collection periods it would be easier to conclude the effect of granite on particle distribution of faeces, but since the significance varies and does not exists on day 13-18, it is hard to conclude the effect of granite. Thus, in the present study, granite had some significant effect on particle distribution of faeces, at some given ages, but the overall effect of granite is rather unclear, as it is rather negligible.

Even though the data for day 11-13 and 13-18 not were corrected for grit when calculating the particle distribution the results still give a good picture of the distribution. The grit in each sample was about 1 gram/treatment, and this gram was distributed on the different sieves, thus hopefully this small amount did not affect the particle distribution of the faeces particles to a very high extent. The significant higher proportion of faeces particles between 0.8 and 1.4 mm on day 11-13 might be explained due to disturbance from potential granite since the data was not corrected for the granite particle distribution, but this explanation does not explain why granite had a significant higher proportion of faeces particles between 0.8 and 1.4 mm on day 18-21 since this data was corrected for the granite particle distribution.

5.6 Effect of grit content on empty gizzard

A high correlation between empty gizzard weight and grit content states that there is an interaction on gizzard development due to the grit retained in the gizzard. In the present study, there was no effect on empty gizzard weight from either grit or whole wheat.

The findings from the present study were consistent with findings from Bethke and Kennard (1926). The authors carefully exanimated gizzards of birds fed granite and no grit and found no correlation between the development of the gizzard muscle on firmness and thickness independent on granite or no grit, and concluded that necessity of grit for growing chickens were based on theory rather than facts. These results are contrary several authors; Spencer and Jenkins (1963) found a high correlation between gizzard weight and grit. As previously mentioned, the amount given granite in the present experiment might have been too low to see an effect of granite on gizzard development.

6 Conclusion

As there was no clear and consistent significance of the granite treatment in this experiment, it is not possible to come with a clear and direct conclusion regarding granite addition for broiler chickens. The present study concludes that granite grit does neither improve nor impair broiler chickens performance or gizzard development, except for a significantly higher empty gizzard weight at day 13. Even the combination of grit and whole wheat after day 18 did not seem to affect any performance parameters or gizzard characteristics in the chicken.

As mentioned, literature shows various results regarding performance, gizzard growth, health and economic factors. There is a possibility that todays' highly advanced feed technology and breeding have made granite, as a feed utilization agent, unnecessary for poultry. From a welfare point of view, grit may be beneficial as the bird get the possibility to perform their natural seeking and curious behavior, and the chickens showed a generally high intake of the amount granite offered, thus indicating a preference for granite. Therefore, by offering granite chickens might get the chance to meet an instinct that is desirable for them to preform, and thus alleviate stress. It can also occupy the chickens, and thus enriching their environment. All of these factors may be positive in modern broiler industry, as the birds live in stressful conditions.

There is a possibility that the potential effect of granite is not sufficient to overcome the effect of modern breeding and feed technology, and thereby is indifferent in modern poultry industry.

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