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The Effect of Gritstone Supplementation on Performance and Digestion in Broiler Chickens

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Abstract

A study was conducted to investigate the effect of grit supplementation for broiler chickens' performance and digestion. Three field trials were performed to observe the effect of insoluble quartz grit under commercial conditions. In addition, an experiment under controlled conditions was performed, where the effect of different types of gritstones (quartz, zeolite and marble) were examined for a pelleted diet with and without whole-wheat.

For trail 1 and 2; 6000 day-old as-hatched broiler chickens were divided in to two groups by a solid partition (height 0.5 m). From 3 to 7 days of age, treatment birds got access to quartz grit, in total for trial 1: 9.5 g per bird and 7.5 g for trial 2. The grit was sprinkled on top of the feed. The birds got commercial diets, but diets varied between each trial. Birds samples were taken at day 9 and 28, and 100 gizzards from each group were collected for further analysis. For the third trial, a larger facility was used, where 25 800 day-old as-hatched broiler chickens were equally distributed in two groups. The experimental design was similar to trial 1 and 2, except that 6.9 g grit per bird was given, and the second bird sampling was conducted on day 33.

For the experiment at research facility, 252 day-old male broiler chickens were randomly placed into four equal sized pens. At 5 days of age, 4 birds from one pen where randomly selected and placed in one quail cage, this was repeated 12 times for each pen. Gritstones were given to their respectively treatment group from day 5 to 9 days of age (9.5 g/bird), and from 18 to 20 days of age (3 g/bird). The birds were given commercial diets. From 18-21 days of age, the remaining birds got access to a mixed diet consisting of 15% whole wheat and 85% starter diet. One randomly selected bird from each cage was killed and dissected on day 13, 18, 21.

Feeding insoluble grit showed no convincingly effect on feed conversion ratio (FCR), weight gain, apparent metabolizable energy (AME), nor did it affect pH level in gizzard content, gizzard development and gizzard lesions. However, it seems that insoluble stones do aid in particle reduction to a certain degree, most evident when whole wheat was included in the diet. Use of calcific stones should be used with care and should not be recommended in use for broiler chickens, since it may influence and disturb the birds' mineral balance, thereby reduce the bird's performance.

Key words: Gritstones, gizzard development, broiler, performance, digestion, gizzard lesions

Sammendrag

En studie ble utført for å undersøke effekten av kråsstein på slaktekyllings fordøyelse og dens produksjonseffektivitet. Tre feltforsøk ble utført for å observere effekten av kvartssand under kommersielle forhold. I tillegg ble et eksperiment utført under kontrollerte betingelser, hvor effekten av forskjellige typer kråssteiner (kvarts, zeolitt og marmor) ble undersøkt for en pelletert diett, med og uten hel-hvete.

For feltforsøk 1 og 2 ble 6000 dag-gamle kyllinger delt in to grupper, med en delt vegg mellom seg (høyde 0,5 m). Fra 3 til 7 dager fikk kråsstein gruppen tilgang til kvartssand, totalt 9,5 g pr. fugl og 7,5 g pr. fugl for feltforsøk 2. Kråssteinen ble drysset jevnt på toppen av fôret. Fuglene fikk kommersielle dietter, men diettene varierte mellom hvert forsøk. Prøver av fugler ble tatt på dag 9 og 28, og 100 kråser fra hver gruppe ble oppsamlet fra slakteriet, for videre analyse. For det tredje forsøket ble et større anlegg brukt, hvor 25 800 dag-gamle kyllinger ble fordelt likt i to grupper. Forsøksdesignet var likt som i feltforsøk 1 og 2, bortsett fra at 6,9 g sten ble gitt pr. fugl, og den den siste dagen for prøver ble gjennomført på dag 33.

For forsøket på forskning anlegget, ble 252 dag-gamle hann-kyllinger tilfeldig plassert i fire like store bur. Ved 5 dagers-alder ble 4 fugler fra et bur tilfeldig valgt, og plassert i et vaktel bur. Dette ble gjentatt 12 ganger for hvert bur. Kråsstein ble gitt fra 5 til 9 dagers alder (9,5 g / fugl), og fra 18 til 20 dagers alder (3 g/fugl). Kommersielt fôr ble gitt til alle fuglene. Fra 18-21 dagers alder fikk de resterende fugler adgang til en blandet diett bestående av 15% hel hvete og 85% start fôr. En tilfeldig valgt fugl fra hvert bur, ble slaktet og dissekert på dag 13, 18, 21.

Fôring med uløselig kråsstein viste ingen overbevisende effekt på fôrfaktor (feed conversion ratio, FCR), tilvekst, omsettelig energi (apparent metabolizable energy, AME), og påvirket heller ikke pH-nivået av innhold i kråsen, kråsutvikling, og kråssår. Imidlertid kan det virke som uløselige kråsstein bidrar til partikkelreduksjon av fôrmaterialet, mest tydelig når hel hvete ble inkludert i dietten. Bruk av kalkrike stener bør brukes med forsiktighet, og bør ikke anbefales i bruk som eneste form for kråsstein til slaktekylling, siden det kan påvirke og forstyrre fuglens mineral balanse, og dermed redusere dens ytelse.

Stikkord: Kråsstein, kråsutvikling, produksjonseffektivitet, fordøyelse, kråssår

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1. Introduction

The poultry industry has changed dramatically over the last decades. The increased demand for cheap and high-quality protein has been one of the driving forces. Advances in nutrition, housing conditions and most importantly, animal breeding have contributed to create one of the most intensive production systems for domestic animals.

The poultry breeds of today have an extreme potential for productivity compared with those used 60 years ago (Havenstein et al. 1994; Havenstein et al. 2003; McMillan et al. 1990; Wheeler & Campion 1993). A modern layer produce approximately 363 eggs in 56 weeks and male broilers are already reaching a live weight of 2 kg by day 33 (Aviagen 2014; Lohmann Tierzucht 2012). This is largely due to improvements in the genetic makeup of the bird. However, to fulfil the genetic potential of the bird, both nutritional knowledge and management are of great importance. As seen by Havenstein et al. (2003) 10% to 15% of the differences in carcass yield are explained by nutritional factors.

In the past, the main aim for nutritionists has been to exploit the bird's genetic potential for productivity. Consequently, over-formulating of diets, especially for critical elements have been common (Ravindran 2012). Whereas today, increased focus on animal health, product quality and sustainable feed production is taken more into consideration. For instance, researchers have started questioning the feed efficiency of the modern broiler chicken. How efficient the bird absorb and utilize the nutritious elements in the diet depends on the chemical and mechanical treatment of the feed (Ravindran & Abdollhi 2014), and the amount of antinutrients present, i.e., soluble fibre (Choct et al. 1996). In addition, factors regarding the bird itself may influence the digestibility. In 2001 Svihus & Hetland proposed that commercial broiler chickens fed ad libitum would overconsume feed, thereby reducing digestibility. Research by Cline et al. (2010) and Lacy et al. (1985) strengthens this observation. Their results showed that broiler strains selected for high growth rate and appetite had less sensitivity to satiety signals, thus being less able to control feed consumption, compared to the lean strains.

Undigested material is of environmental concern due to pollution and the limited feed resources available. Moreover, it influence both directly and indirectly bird's performance by 1. Stimulating gut microbiota growth and activity in the posterior digestive tract, thereby reducing the digestibility even further (Choct et al. 1996). 2. Enlarging the proportion of undesirable microbiota in the gut, such as *Clostridium perfringens*, hence increasing the risk of diseases like necrotic enteritis (NE) (Kaldhusdal & Hofshagen 1992). 3. Causing a higher degree of wet litter and sticky droppings (Kaldhusdal & Hofshagen 1992). This being particularly relevant today, where the feed industry is trying to find good alternatives to replace antibiotic growth promoters. In 2014, an incidence of resistant bacteria in chicken meat in Norway had a dramatic effect on the Norwegian's consumption of poultry meat. Concerns regarding the possible link between the ionophore coccidiostats and the presence of resistant bacteria in chicken meat (VKM 2015) have led to that the Norwegian feed industry is gradually phasing it out of production. Improving the birds' feed efficiency is therefore of utmost importance.

Despite the fact that the modern broiler chicken is not able to control the feed flow as efficiently as in the past, adding structural components in the diet may compensate for this effect by stimulating the functionality of the gizzard (Svihus 2011). A well developed and stimulated gizzard has been seen to increase digestibility (Amerah et al. 2009; Hetland et al. 2003; Rogel et al. 1987; Svihus & Hetland 2001), improve AME (apparent metabolizable energy) (Amerah et al. 2009; Svihus & Hetland 2001) and gut health in poultry (Amerah et al. 2009; Bjerrum et al. 2005; Jones & Taylor 2001; Riddell 1976).

Enhancing the digestive function of domestic birds by giving them access to structural components is not a revolutionary thought in the field of nutrition, already studied intensively from the beginning of the 20th century (Gionfriddo & Best 1999). Several studies showed beneficial effects of gritstones on layer and broiler chicken performance (Balloun & Phillips 1956; Scott & Heuser 1957; Smith & MacIntyre 1959), and use of gritstones have been recommended to increase the economic output in the production (McIntosh et al. 1962). On the other hand, some studies have shown no or little effects (Svihus et al. 1997). In addition, there have been raised concerns on overconsumption followed by impaction of gritstones in the crop and gizzard (Macwhirter 2009). Today, the main nutritional research on structure has focused on inclusion of non-soluble fibre (NSP) (Bjerrum et al. 2005; Hetland & Svihus 2001; reviewed by Singh et al. 2014) Nevertheless, the use of grit for broiler chickens has got renewed interests, particularly in Norway because of the present situation mentioned above.

In which degree inclusion of gritstones affect the bird's performance, may depend on feeding regime, grit characteristics and diet-stone interaction. A deeper understanding of these factors in the modern poultry breeds is necessary to utilize the potential of using gritstones today. The aim of this thesis was to investigate if there is a potential of supplementing gritstones to enhance digestion and performance of the modern broiler chicken. Three field trials and one experiment under controlled conditions were performed to test the hypothesis that gritstones stimulates the gizzard organ, thereby increases its functionality. A well-functioning gizzard should be better able to control the feed flow, synchronizing the digesta with the flow of secreted endogenous enzymes in the small intestine. Thus, enhancing the absorption and thereby elevating bird performance. In addition, it was postulated that an increased retention time of gritstones would reduce the pH level gizzard content.

2. Literature

2.1 Peculiarities with the digestive system of the avian species

Birds are one of the few species that lack teeth for mastication, but due the two distinctive organs; the crop and gizzard, it does not influence their performance. On the contrary, birds have been associated with a high metabolic rate (Farner 1960), and they have better feed conversion ratio (FCR) compared to both swine and ruminants.

The crop – the birds' storage organ

The role of the crop as a digestive organ is mainly to be a reservoir for feed particles before the chemical and mechanical digestion in the gizzard. However, the crop does also play a part in preparing the feed particles for further digestion, by softening the material available in the segment. There has also been observed a reflux of feed from the gizzard back to the crop (Duke 1986). Thus, some of the feed particles found in this segment may already have been exposed to the digestive juices and continuing to undergo the degradation processes. A small degree of microbial activity is also known to occur in the crop (Guan et al. 2003), which then, in turn, may be beneficial for exogenous enzyme activity in the crop (Simon & Igbasan 2002). The content in the crop is emptied by peristaltic movements into the proventriculus, where the material comes in contact with gastric juices (pepsin, HCl, and mucus) before entering the gizzard (Duke 1986).

The gizzard – "the gastric mill"

The gizzard is a highly efficient organ composed of two pairs of opposing muscles that grind down feed particles to a critical size before they enter duodenum (Duke 1986), figure 1. In fact, the efficiency of the particle reduction in the gizzard has been compared with the effect of rumination in sheep (Moore 1999). The gizzard do also play a major role in regulating digesta flow through the digestive tract, making sure an even flow of nutrients to the intestines. Tough, gastro-duodenal refluxes may return some of the material back to the gizzard. The gizzard is also considered to influence feed intake (Svihus 2011). In spite of the gizzard lacking mucus secreting glands, it still functions as the primary site of preliminary proteolysis. Rhythmic contractions of the gizzard muscles act as a mixing agent to blend in the digestive juices and feed particles that arrive from the proventriculus, thus compensating for the short retention time in the latter segment (Hetland et al. 2002). A well-functioning gizzard is, therefore, not only important for particle reduction but for several aspects in the digestive processes.

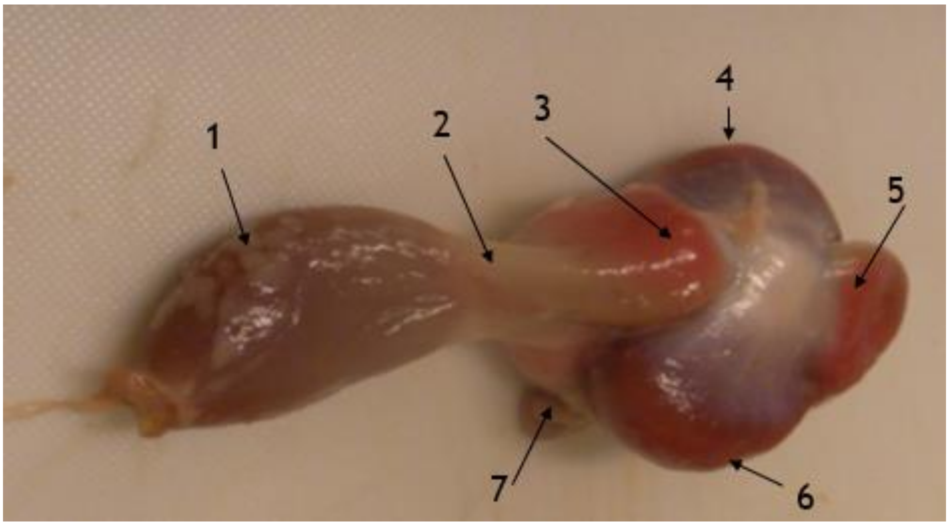

Figure 1: Simplified description of the stomach of domestic chicken 1: Proventriculus, 2: Isthmus, 3: Craniodorsal thin muscle surrounding the cranial sac, 4: Caudodorsal thick muscle, 5: Caudoventral thin muscle surrounding the caudal sac, 6: Cranioventral thick muscle, 7: Pyloric part ending out to the duodenum. Photo: Kari Borg, Based on the description from (McLelland 1979)

The grinding process in the gizzard

Gritstones are generally not thought as essential for bird survival (Gionfriddo & Best 1999). Although, there has been reported that some birds do consume other hard elements to compensate for the lack of stones (Jenkinson & Mengel 1970). Nevertheless, the fact that birds do manage relatively well without grit is due to the gizzard morphology and its grinding cycle. As mentioned earlier the gizzard is composed of two pairs of opposing muscles, two thin muscles placed anterior and posterior according to the long axis of the gizzard, and two thick muscles opposing each other in the lateral direction (Svihus 2011). The slight asymmetrical organization of these muscles is what makes the grinding organ so efficient, causing a translational movement instead of just a compressional force in the gizzard lumen, thereby creating more damage of the feed particles (Moore 1998a). In herbivore birds, the koilin layer (the inner lining of the gizzard muscles) do have a particularly hard consistency and may vary in thickness, being especially thick in the areas opposite the two large muscles (McLelland 1979). These areas are often called “grinding plates” and are more pronounced in some bird species, for example in the domestic chicken. The two grinding plates are similarly asymmetrical to the thick muscles, respectively, making the gizzard lumen particular narrow during contraction (McLelland 1979). Moore (1998a) has described the grinding movement in thorough detail. Only the principle of the grinding cycle will be described here, mostly based on the description by Duke (1992) cited by Svihus (2011). The process of grinding starts by the contraction of the thin muscles. Material in the sacs will be pushed into the gizzard. The pylorus then opens and the material flow into the duodenum by peristaltic movement in the latter. At the same time, material will enter the proventriculus. Immediately after that, the thick muscles will contract. The translational movement occurs when the grinding plates approach each other, at the same time as they move sideways across each other (Moore 1998a). During this action, some of the material will be squeezed out from between the grinding plates into the sacs in both directions (caudal sac and cranial sac) (Moore 1998a). In addition, some material from the anterior part of the gizzard would enter the proventriculus and the duodenum (Svihus 2011). The thin muscles will then contract, pushing material back between the grinding plates (Moore 1998a). Material from the proventriculus will at the same time be returned to the gizzard (Svihus 2011). This process will continue until the food particles are small enough to enter the duodenum (Moore 1998a).

A well developed thick gizzard muscle will implement a larger force towards the grinding plate, than a less developed muscle. A significant larger empty gizzard weight has been recognized for birds given structural components in the diet, i.e., whole grains or hulls (Bjerrum et al. 2005; Hetland et al. 2003). This is because these particular components are retained in the gizzard for longer before they are dissolved, or broken down into smaller particles. This will force the muscles to contract for longer, and as a result, the gizzard muscles will become larger and more developed. A similar trend has been seen for an increase in the gizzard lumen (reviewed by Svihus 2011). Moore (1998a) pointed out that the amount of material in the gizzard, the size and narrowness of the gizzard lumen is equally important for an efficient grinding. In fact, a large lumen volume will increase the amount of particles present in the gizzard, but will in turn decrease the efficiency of the grinding plates, because it will limit the translational movement by physically preventing the grinding plates contacting each other.

Ingested gritstones will affect the grinding process in the gizzard in a positive or negative way, depending on the amount eaten and the characteristics of the stones. By adding grit with an angular surface, the stones will contribute to the shear force on the feed particles, as seen on the breakdown of grass in geese by Moore (1998b). The same author observed that well-rounded stones had less or almost adverse effect on grinding, compared to angular stones, mainly taking up space in the gizzard. Consequently, it will then reduce the potential space for feed particles. Moreover, too large quantity will limit the action of the thick grinding muscles during the contraction cycles of the gizzard. The material is in a way too tough/hard to squeeze together.

Mixing of the feed particles may also be an important effect of gritstones. In ostriches (*Struthio camelus*) gritstones are believed to prevent formation of grass agglutinates followed by constipation of material in the pyloric region (Wings 2004). The ostriches are mainly feed on grass, thus the relevance of the latter may be of less importance for commercial chicken production. Although, a longer retention time in the gizzard combined with a mixing effect could potentially expose the feed particles for chemical digestion for a longer period of time.

2.2 Use of gritstones - perspective

Consumption of grit are known to occur in several bird species (Gionfriddo & Best 1999; Wings 2004). However, this is not an extraordinary practice among animals. Stomach stones are also reported to be present in marine mammals and reptiles, like sea lions (*Otaria byronia*) and crocodiles (*Crocodylus porosus*) (Taylor 1993). Grit usage has been found in several extinct species dating back to the dinosaurs (Wings 2004), suggesting that ingestion of stone fragments must have been an evolutionary wise strategy for some animals. Extensive reviews have been done on gritstone consumption for avian species (Gionfriddo & Best 1999), dinosaurs and extant bird species (Wings 2004) and marine tetrapods (Taylor 1993).

Although several theories are postulated regarding the purpose of deliberate ingestion of stone fragments, the most plausible explanation, generally accepted by the scientific community is that the stones are consumed to enhance the digestive function in the animal (Wings 2004), this being most evident for bird species (Whittle & Everhart 1993). There has also been shown that some bird species without access to grit may die of starvation (Wacquant-Geozelles, 1892; Short. 1993:35, cited by Gionfriddo & Best 1999) although this is considered to be rare and only in extreme cases (Gionfriddo & Best 1999). The function of gritstones is mainly thought to benefit the digestive system by either contributing to a better grinding and mixing of the feed particles, by stimulating the excretion of digestive juices, and or by supplying the animals with minerals (Gionfriddo & Best 1999; Wings 2004).

The occurrence of gritstones is particularly evident for herbivore birds, compared to both omnivore and carnivore species (Gionfriddo & Best 1999). This is likely due to the differences in their natural diets. Herbivores do consume a higher amount of hard components that are resistant to chemical and mechanical breakdown, thus gritstone consumption would be more beneficial for these species. This do also correspond to the difference in characteristics of the birds grinding organ - the gizzard, being more developed and enclosed organ in herbivores as opposed to being more oval and less distinct shape in carnivores (McLelland 1979). The most important commercial poultry species today are within the group of *Galliforms*. These birds should be classified as omnivores, due to the varied diet of the ancestors (i.e., *Gallus gallus*) (Klasing 2005). However, Svihus (2011) pointed out that these birds, in fact, do have a particular well-developed gizzard that will benefit from structural influence. Also, the diet for today's poultry (ie. broiler chickens, layers, quail, and turkeys) do mostly consist of plant-based material. For commercial use, the adding of gritstones has been thoroughly investigated and supplied to domesticated birds from the middle of the 20th century (Gionfriddo & Best 1999). However, it is not a currently common practice today, at least not for commercial broiler production. For layers, on the other hand, soluble grit is used more regularly. Although, this is probably most due to the need of fulfilling the bird's high requirement of calcium rather than improving the feed efficacy per se.

For the slaughterhouses, gritstones could potentially be a challenge for the equipment if birds have stones retained in their gizzards at the time of slaughter. However, according to the project manager at Lantmännen, Danpo, this is not currently an issue in Norway since the whole digestive tract is removed and not utilized further (Hylleberg 2016, pers. comm., 13. April). In Denmark, on the other hand, the gizzards are collected for human consumption (Hylleberg 2016, pers. comm., 13. April), thus gritstones may wear down the knives, consequently result in a more frequent replacements of equipment.

2.3 Gritstones characteristics

"The avian teeth" has been a common name on gritstones, in relation to the grinding effect of the stones in the gizzard. A more official definition is set by Gionfriddo and Best (1999) as follows:

"Gritstones are stones and rock fragments that are ingested by birds, excluded very fine particles such as dust, ash, and clay".

Universally accepted, a gritstone has to be of minimum 0.065 mm diameter to not fall under the latter categories (Wings 2004). The definition by Gionfriddo and Best (1999) is quite broad and includes stones with variations in shape, size, colour and chemical composition. These factors will influence both the amount of stones digested, the retention time and the physiological effect on the birds.

There are two main group of gritstones; insoluble or soluble stones (Smith & MacIntyre 1959). Insoluble grit (i.e., granite, silica sand and quartz) are resistant to the acid degradation in the gut, whereas soluble grit (i.e., marble, limestone) will easily degrade in a moist and acidic environment. Soluble stones will contribute more to the bird's mineral requirement. Furthermore, calcific gritstones may affect the gastric environment by increasing the pH. An elevated pH may reduce endogenic enzyme activity (Piper & Fenton 1965) and be beneficial for pathogenic bacteria in the digestive tract (Bjerrum et al. 2005). The chemical composition should, therefore be taken into account when formulating diets, since the supply of minerals can exceed the birds' requirements, with possible toxic effects. Itani (2015) noted that if feeding Dolomite Grit with an amount of 15 g per bird to broiler chickens, it would supply the birds with a magnesium level 11.6 times larger than required. Without even including the magnesium level in the diet, the requirements exceeded the toxic level of magnesium.

The shape of grit can vary from a spherical to an oblong form, with variations in surface texture ranging from an angular to a smooth surface (Smith 1960). Gionfriddo (1994) observed that the majority of grit sampled from House Sparrow (*Passer domesticus*) had a partially smooth surface. However, care must be taken when evaluating the bird's preference for both grit shape and size due grit being exposed to abrasion forces by the erosive environment in the gizzard (Gionfriddo & Best 1999). Itani (2015) illustrated this by comparing initial gritstones (as-fed to birds) to grit found in the gizzard. There was a marked difference, where the grit in the gizzard had a more rounded surface, compared to the initial fed grit, which was more angular in shape.

Variation in grit size has been observed in between and within several wild species (Beaune et al. 2009; Best & Gionfriddo 1991). Beaune et al. (2009) found gritstones varying from 0.05 cm to 2.2 cm in King Penguin chicks (*Aptenodytes patagonicus*). In several species, a linear relationship has been observed between grit size and $\log_{(10)}$ of the birds' weight (reviewed by Gionfriddo & Best 1999).This must be taken into account when giving grit to our poultry species, for example, the optimal size range of grit given to turkeys may differ from what should be given to broiler chickens. Although, it may not be detrimental to performance the stone size may limit potential benefits of gritstone addition (McIntosh et al. 1962). This may also be relevant concerning grit given to young versus older birds. As seen by Alson (1985) (cited Gionfriddo & Best 1999) increasing age would result in a higher number of large particles being consumed.

2.4 Effect of grit on digestion and performance

The vast amount of papers examining the effect of gritstones on digestion and performance are far from consistent in their results. Already early on, scientists questioned if gritstones were indeed necessary elements in poultry nutrition. Bethke and Kennard (1926) claimed that the potential of supplementing gritstones for aiding the grinding process in the gizzard were non-existent and that the general views on gritstones was largely based on traditions and assumptions, rather than scientific evidence.

"The belief that git is essential in the gizzard for grinding or reducing purposes in the case of growing chicks appears to be based on theory rather than facts" - Bethke and Kennard (1926)

Contrary to the statement from Bethke and Kennard (1926), most authors are less conclusive regarding the effect of gritstones. Hetland et al. (2003) found that particle size of digesta in the duodenum was significantly reduced ($P<0.05$) when birds got access to grit. This indicating that the gritstones do in fact aid in the grinding process. However, the authors did not find any improvements in ileal starch digestibility related to the ingestion of gritstones. Significant improvements have by been observed for metabolizable energy (ME) (Evans et al. 2005; McIntosh et al. 1962; Smith & MacIntyre 1959), digestibility (Smith & MacIntyre 1959; Smith 1960) increased weight gain (Balloun & Phillips 1956; Scott & Heuser 1957) and feed efficiency (Evans et al. 2005; Scott & Heuser 1957). Other scientists have only found slight improvements (Fritz 1937; Jull 1915; Rowland & Hooge 1980; Spencer & Jenkins 1963), whereas some have not found any effect at all by adding gritstones to the birds feed ration (Bennett et al. 2002; Bennett & Classen 2003; Garipoglu et al. 2006; Itani 2015; Svihus et al. 1997; Taylor & Jones 2001; Waldenstedt et al. 1997). Tepper et al. (1939) reported that the beneficial effect of gritstones on feed efficiency was first noticeable after four weeks.

Some studies have found adverse effects on performance. Arscott et al. (1955) observed slightly lower live weights for New Hampshire chicks fed on grit, compared to those not having access to gritstones. Majewska et al. (2009) observed that turkeys fed on silica grit had the highest mortality rate, reaching 11.1% in a 20 week period, compared to the diets with similar inclusions level of charcoal (4.4%) or hardwood ash (6.6%), or up against the control (8.8%).

The effects of gritstones on digestibility and performance vary in relation to the stone characteristics. For instance, Heuser and Norris (1946) found detrimental effects on the growth rate of giving 5% calcite grit to White Leghorn Cocks, while this was not observed for the birds fed granite grit. The same authors also investigated the effect of force-feeding the birds with grit. A slight advantage was seen for the granite fed group while the birds that were forced to eat calcite consequently had a lower feed consumption and growth rate. In the study of Smith and MacIntyre (1959) both limestone and quartz were used. Both types of grit showed a beneficial effect on digestibility and ME. However, they did influence the digestible fragments to a different degree. For instance, limestone improved the ether digestibility, while quartz influenced crude protein, fibre and N-free digestibility.

A couple of studies have also examined the differences between insoluble grit types. Balloun and Phillips (1956) examined the effect of grey granite, red quartzite granite, and common river sand, and found no differences between stone types. Scott and Heuser (1957) observed a slight difference in preferences between the two types of insoluble grit (granite vs. feldspar), towards the feldspar grit. In addition, the authors did see a reduction in soluble grit intake if the birds also had access to insoluble grit.

Smith and MacIntyre (1959) observed slightly better improvements in ME for smaller sized stones (sand) versus larger particles (hen-sized grit). Similarly, McIntosh et al. (1962) found no effect on ME by using hen-sized grit, whereas supplying the chickens with smaller gritstones (grower sized grit) did significantly increase the ME values. Conversely, Fritz (1937) found a tendency for a higher feed efficiency when the turkey-sized grit (6-14 mm) was given to the birds, compared to when a finer grit type was used, “baby” sized grit (average 1 mm).

The coarseness of the diet will vary with inclusion of different structural components like, whole wheat, oat hulls, etc. Also, feeding a mash diet versus a pelleted diet may reduce the level of large particles in the diet (Svihus et al. 2004). Diet-gritstones interactions have been observed for digestibility and performance (Evans et al. 2005; Jull 1915; McIntosh et al. 1962; Smith & MacIntyre 1959), gritstones being more beneficial for coarser diet. For instance McIntosh et al. (1962) observed that the ME value was higher for diets with whole wheat, compared to either if the grains were pelleted or ground. However, other studies do not support this view (Bennett et al. 2002; Bennett & Classen 2003; Hetland et al. 2003; Svihus et al. 1997). These studies show that gritstones did not influence feed efficiency with either inclusion of whole grain or hulls in the diets to broiler chickens or turkeys.

2.5 Effect of grit on gut health and microflora

Nutrition, gut morphology, gut flora and health status of the bird are closely intertwined with each other, and will all influence the bird performance and economic output of the production.

As described earlier, the structural components like whole grains and hulls will affect the gizzard morphology due to increased retention time in the organ (Bjerrum et al. 2005; Hetland et al. 2003). Similarly, increased gizzard weight has been observed for gritstones (Garipoglu et al. 2006; Hetland et al. 2003; Heuser & Norris 1946; Scott & Heuser 1957). Although, some authors have not found this effect by supplying the birds with grit (Bennett et al. 2002; Jones & Taylor 1999). Structural components have also been seen to affect the development of the proventriculus, making it more distinguished from the gizzard compartment and reduce dilation scores (Jones & Taylor 1999; Taylor & Jones 2001).

The microflora in the digestive tract of animals is complex; the avian species are no exception. The microbial population consists of both beneficial and pathogenic bacteria. They are mainly present in the ceca but can also be found further up in the intestines to the crop (Apajalhti & Kettunen 2006). As already mentioned, microbes in the small intestine will compete with the bird's digestive mechanisms for available nutrients. Undigested material in the small intestine will benefit the microbes, giving them optimal growth conditions (Choct et al. 1996; Kaldhusdal & Hofshagen 1992). The pathogenic bacteria will thrive and cause intestinal necroses and reduction in the birds' performance (Kaldhusdal & Hofshagen 1992).

The increased retention time of feed particles and increased development of the gizzard organ has been linked to a reduction in pH (Svihus 2011). For instance, Bjerrum et al. (2005) observed a significant decrease in pH of gizzard content when the diet was supplemented with whole wheat, as opposed to the same amount of ground wheat (P=0.003). The reduced pH will be beneficial for endogenous enzyme activity (Piper & Fenton 1965), thereby reducing the available nutrients for microbes in the gut. In addition, like enzymes, the microbes have a tolerance for a certain pH level. A pH level outside this threshold would cause less optimal growth conditions for the microflora. Reduction in bacteria population, such as *Salmonella Typhimurium, Clostridium perfringens, Lactose-negative enterobacteria,* amongst others, have been found with the inclusion of whole grain in the diet (Bjerrum et al. 2005; Engberg et al. 2004). However, Waldenstedt et al. (1997) found no benefit of diet structure (whole grain or gritstones) on birds infected with the protozoan parasites *Eimeria tenella* and *Eimeria maxima.* Gabriel et al. (2006) found even adverse effects on the clinical health status of birds by feeding a diet where whole grain was included. In the past, there were raised concerns regarding if increased development of the gizzard together with the abrasive effect of gritstones could increase the hatchability of embryonated parasite ova. However, Riedel (1950) found no such effect for chickens infected with *Ascaridia galli* ova.

Influence of structural particles has been seen shown to increase the gastro-duodenal refluxes (Hetland et al. 2003), which in turn may improve gut health. Bile acids have been observed to reduce the incidence of gizzard erosion and lesions in chickens (Almquist 1938b). As well as being a welfare and an ethical issue in poultry production, gizzard lesions have been found to explain 31.8% of the variation in *Clostridium perfringens* counts in the gizzard (Novoa-Garrido et al. 2006). In addition, the authors observed a significant positive correlation between the severity of the gizzard lesions and *Clostridium perfringens* counts. The authors did not manage to provide any explanation of the causes of the gizzard lesions. In a review on causal or predisposing factors, one risk factor postulated was, in fact, *Clostridium perfringens* (Gjevre et al. 2013). The bacteria do hydrolyse bile salt, thus reducing the protective effect of bile in the gizzard. Novoa-Garrido et al. (2006) did observe clinical signs of NE without the presence of gizzard lesions, which strengthens the possibility that *Clostridium perfringens* may be one of the causing agents.

The effect of grit and grit like substances on the koilin layer have been shown to reduce the frequency and severity of gizzard lesions (Almquist 1938a). Although, the same author observed an effect of grit size, where fine grit showed no effect on the koilin layer compared to birds receiving no grit. Bird et al. (1937) stressed that the gritstones may have different effect on various types of lesions (ie. crater lesions versus spongy material). In fact, the author proposed that the grit may have an adverse effect on crater erosions if the lesions are already present at hatch.

3. Material and Methods

Three field trials were carried out at Tau, Rogaland, Norway, between the 2nd of November 2015 and the 16th of January 2016. In addition, an experimental trial was conducted in the Animal Production Experimental Centre, at the Norwegian University of Life Sciences (NMBU), Ås, Norway, between the 12th of November to 4th of December 2015. The experiment at NMBU was a part of a comprehensive study, where the effect of different types of gritstones and the interaction between whole-wheat and grit were examined.

3.1 Gritstones

Quartz Grit

The quartz grit was purchased from Sibelco Nordic AB, a supplier of industrial minerals. The gravel was produced at Woldstad Sandforreting in Norway, with a dimension of 2.0 to 3.5 mm. The same quartz grit was used in the field trials as in the experiment carried out at NMBU. The chemical composition of the quartz stones is shown in Table 1.

Quartz has a hardness of “7”, given by Mohs scale of hardness (Oftedahl 1980).

Table 1: Average values for the chemical composition of quartz grit (Sibelco Nordic Sibelco n.d.)

SiO2	Silicon Dioxide	79.50 %
Al2O3	Aluminium Oxide	9.57%
K2O	Potassium Oxide	3.62%
Na2O	Sodium Oxide	2.55%
Fe2O3	Iron (III) Oxide	2.04%
CaO	Calcium Oxide	1.66%
MgO	Magnesium Oxide	0.67%
TiO2	Titanium Dioxide	0.28%

Zeolite Grit

The zeolite grit with 1 mm to 2.5 mm dimension was ordered from ZEOCEM. The chemical composition of the zeolite is shown in Table 2. The lab result analysed 34 types of different minerals and only the main elements are shared here.

Zeolite is categorized as an insoluble mineral but is softer than granite and quartz. The hardness does vary for different zeolites, but the hardness of stones used in this trial, given by ZEOCEM is measured to be “1.5-2.5”, on Mohs scale of hardness (Oftedahl 1980)

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

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

Marble

The marble grit, with a stone dimension of 1-2.5 mm was purchased, from Visnes Kalk AS, a supplier of calcific stones in Norway. The chemical composition is shown in Table 3.

Calcific stones have a hardness of "3", given by Mohs scale of hardness (Oftedahl 1980).

Table 3: Average values for the chemical composition of marble grit (Visnes Kalk AS 2007).

$CaCO_3$	Calcium Carbonate	98%
$MgCO_3$	Magnesium Carbonate	1%
Fe_2O_3	Iron(III)oxide	0.1%
SiO_2	Silica	0.6%

3.2 Field trials

Day-old as-hatched broiler chickens (Ross 308), with an average body weight of 45 g were obtained from a commercial hatchery, Hå hatchery (Nærbø, Rogaland, Norway). The chickens were spray vaccinated (Paracox 8) before arriving at the facility. The field trials all consisted of one treatment and one control group. The birds were raised under commercial conditions, divided into two groups by a solid partition (height 0.5 m). Animal density was equal in each section. The control group and the treatment group got identical diets, without coccidiostats. However, diet composition varied between the trials. Particle distribution was therefore performed on each diet, according to the Standard Wet Sieving Analysis Procedure from The Centre of Feed Technology/Fôrtek at NMBU (Miladinovic 2009).

3.2.1 Trial 1

Animal housing

6000 day-old chickens arrived at the chicken house 2nd of November. 3 300 chickens were placed in section 1 (185m^2), while the rest, 2 700 chickens were put in section 2 (150 m^2). A thin layer of rapeseed straw pellets covered the solid concrete-floor. From day one, the lightning programme consisted of a 23 h light period and a 2x1 h dark period. From day five to the end of the experiment the light programme was changed to 2x4 hour dark period (between 24.00-04.00 am; 12.00-16.00 pm). The temperature was 32 ℃ (day 0) and gradually decreased to 20 ℃ (day 28). The birds had access to automatic nipple drinkers with drip cups while feed was given in 48 feed pans, in section 1 and 40 feed pans in section 2. An automatically timer controlled the feeding. The feeding regime was slightly restricted, by letting the birds empty the feeders once a day.

Diet

All birds were given a starter diet (0 to 10 d), a grower diet (10-28 d) and a finisher diet (29-32 d) without coccidiostats. Due to increased level of spores, an observed higher degree of wet litter and reduction in daily gain, it was decided to implement coccidiostats in the diet, thus narasin was included in the grower diet, from day 22 to 28.

Bird experiment

From 3 to 7 days of age, birds in section 1 got access to grit (9.5 g per bird). Grit provision was initiated with 50 g per feed pan, which were raised to 150 g on day 4 and continued to day 7. Grit was measured in two measuring cups (50.01 g SD 3.5;149.4 g SD 1,3) and sprinkled evenly on top of the feed, see figure 2. Observation of grit was done on day 8. At 9 days of age 20 chickens were killed and frozen. Litter samples from section 1 were taken with a collection frame (0.12 m^2), randomly from 6 places excepted of 1 meter from the feeding pans. This to exclude noise from feed spillage. At 28 days of age, 10 birds from each treatment were killed and frozen. The remaining birds were sent to slaughter at day 32. At the slaughterhouse 100 gizzards from each group were collected and frozen down for later analysis.

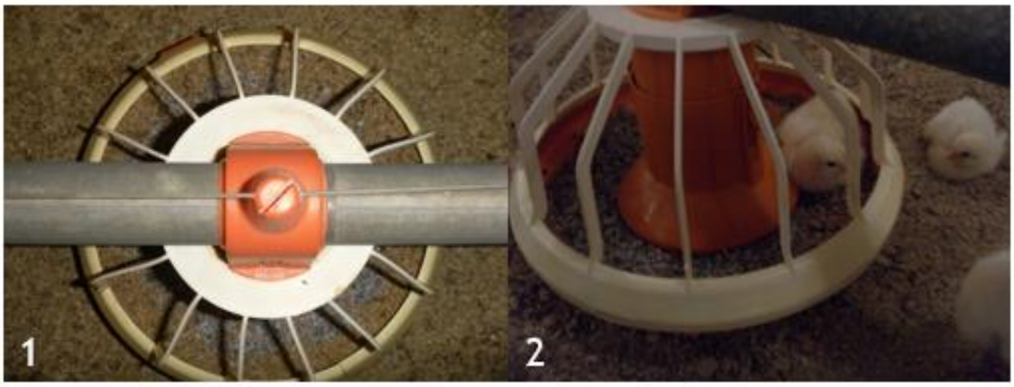

Figure 2: Gritstones were sprinkled on top of the feed, as shown in 1 and 2. Photo: Kari Borg

3.2.2 Trial 2

6000 day-old chickens arrived on the 10^{th} of December and were reared in the same housing conditions as trial one, except the grit fed birds were allocated in section 2, while the control group was placed in section 1. All birds were given a starter diet (0 to 5 d), a grower diet (5-28 d) and a finisher diet (29-34 d) without coccidiostats.

The experimental procedure was the same as trial 1. However, the birds received less amount of grit, 30 g + 120g x4 per feed pan which equals 7.5 g per bird. Gritstones was measured in two measuring cups (31.24 g SD 0.87;121.01 g SD 2.28).

3.2.3 Trial 3

25 800 chickens arrived at 14^{th} of January. The number of birds was divided equally into two sections (700 m^2 x2). From day one, the lightning programme consisted of a 23 h light period and a 2x1 h dark period. A thin layer of wood shavings covered the concrete floor. In addition to radiant heating, the temperature was also adjusted through underfloor heating. From day five to the end of the experiment the light programme was changed to 2x4 hour dark period. The temperature was 32 ℃ (day 0) and gradually decreased to 20 ℃ (day 28). The birds had access to automatic nipple drinkers with drip cups while feed was given in 160 feed pans, in each section. An automatically timer controlled the feeding. The feeding regime was slightly restricted, by letting the birds empty the feeders once a day. All birds were given a starter diet (0 to 10 d), a grower diet (10-28 d) and a finisher diet (29-32 d) without coccidiostats.

The experimental procedure was similar to trial 1 and 2. However, the birds received less amount of grit; 30 g +130g x4 per feed pan which equalled 6.9 g per bird. Grit was measured in two measuring cups (31.24 SD 0.87; 130.09 SD 0.73). Additionally, the last sample day was moved to day 33 due to practicalities.

3.2.4 Dissection

The frozen birds collected during the trials were thawed at room temperature prior to dissection, with the exception of birds collected the last sample day on trial 3. These birds were killed and dissected immediately. Carcass weight for each bird was recorded before dissection. This process was executed in the chicken house, thus for practicalities a digital kitchen scale with 5 kg capacity were used to measure the carcass weight. The proventriculus and gizzard were removed carefully and frozen before transported to NMBU for further analysis. Sartorius AX2202 digital scale with 2200 g capacity and 0.01 g readability was used to measure further weights. At NMBU, the organs were once again thawed, and excessive fat surrounding the proventriculus and gizzard was removed with a blunt knife. The gizzard was detached from proventriculus by cutting the middle of the isthmus. Full weight and the empty weight of the gizzards were recorded. The content from grit-fed birds was collected to find the quantity of grit present in the gizzards.

From the slaughterhouse, 100 gizzards from each group were collected from to find quantitative variation in grit retention. The gizzards were weighed full and empty and content from the grit-fed birds was collected. 72 gizzards from the grit-fed group were randomly selected to find individual differences between birds.

Gizzard lesions were measured for all trials. Gizzards were divided into three groups according to the severity of lesions; healthy, moderate and severe lesions. Lesion smaller than 1 mm were not recorded. The evaluation was based on the paper by Novoa-Garrido et al. (2006), although with a few modifications. The gizzards were scored as shown in figure 3. Before the evaluation, the gizzards were rinsed and put into identical boxes, with treatment identification underneath. The boxes were then shuffled and marked with a letter before evaluation, to ensure randomization. The gizzard from each box were then judged, respectively.

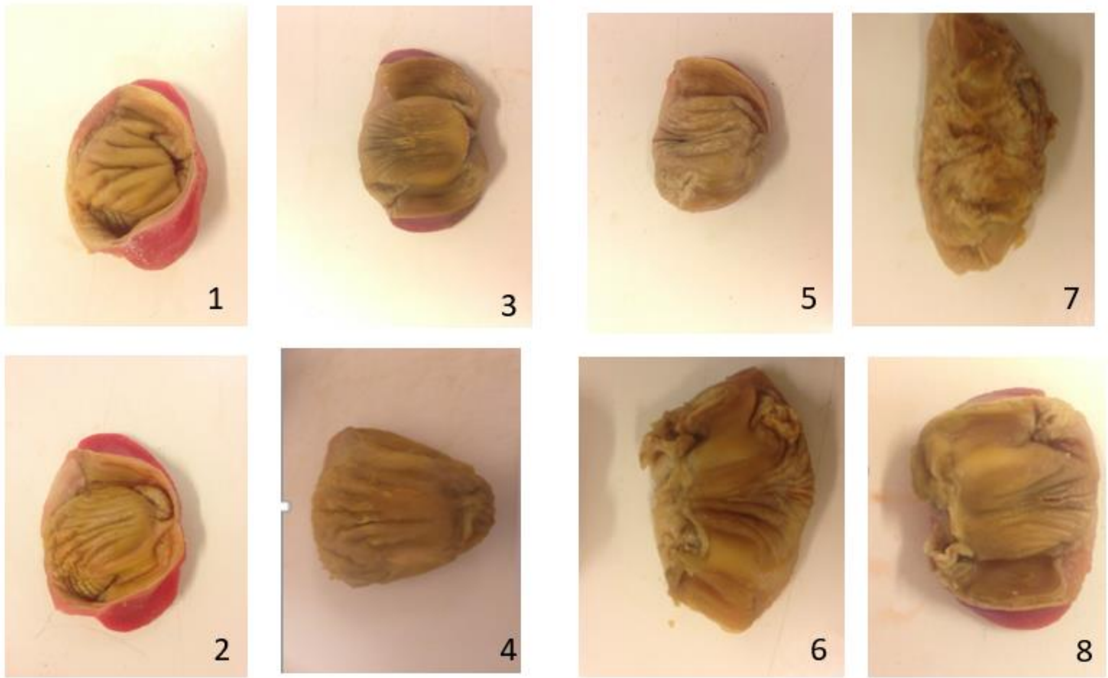

Figure 3: Healthy gizzards: No lesions present. The koilin layer has a smooth surface (1,2). Moderate lesions: Appearance of focal lesions in the koilin layer without the pronounced presence of spongy material (3,4). Severe lesions: Extensive spongy material and or severe puncture/holes in the koilin layer (5-8).

3.2.5 Gritstone analysis

Particle distribution of initial gritstones

Three samples from the original gritstones (approx. 100 g) were sieved to find the actual particle size distribution of grit given to the birds. A Retsch sieve shaker (AS 200 Control) was used, with a set amplitude of 3.0 mm/g and a 3 min sieving time. The eight sieves had a screen opening of 3.55 mm, 3.15 mm, 2.8 mm, 2.5 mm, 2.0 mm, 1.6 mm, 0.8 mm and 0.5 mm. The percentage particle distribution was calculated with the equation shown below (1).

$$\text{\% of particle of nth Size} = \frac{\text{weight of sieve full (g)- weight of sieve empty (g)}}{\text{weight of sample (g)}} \qquad \textbf{(1)}$$

Due to human error, the particle distribution was measured of the remaining gritstones in the bag, after the birds were fed. However, the particle distribution was assumed to be equal in the bag.

Particle size distribution of fed gritstones

The amount of grit in the gizzard were separated from the other particles by the flotation method (decantation), as described by (Itani 2015). The gizzard content was emptied in a bowl of water. The rinsing method consisted of holding a bowl under a tap, letting slow running water through. This disturbed the particles, so the low-density particles floated up and was washed out while the high-density particles, the gritstones, were left in the bottom of the bowl. The gritstones were then dried overnight at room temperature and weighed the following day.

After weighing the individual samples, the gritstones were pooled together for particle distribution, respectively. The same method was used as for the initial gritstones.

3.2.6 Calculations and statistics

Production parameters

Total feed intake was measured by the computer log system in the chicken house. Dead and culled birds were recorded through the whole trial period, and the total mortality was corrected for the number of birds removed for analysis. The average slaughter weight was recorded and given by the slaughterhouse. Average daily gain was corrected for start weight of day-old chicks.

FCR (feed convention ratio) was calculated by dividing feed consumption on weight gain, and correcting for the weight of birds removed for analysis. The birds slaughter weights were added to the total slaughter weight. To find the slaughter weight of these samples, a correction factor was used (Animalia 2013). The bird weights on day 9 were considerably lower than the average live weight of birds used by Animalia (2013). Thus, a linear regression analysis was performed to find the correction factor for the sample birds. The same equation was used on the average weight for the day-old chicks, to find the weight gain for the birds. Statistical software used was R. Equation, and correction factor stated below (2):

$$\text{Correction factor} = 0.0025 \times \text{live weight (g)} + 63.95 \qquad \textbf{(2)}$$

$R^2 = 0.99$; p-value = 5.57e-15

Gizzard evaluation data

Descriptive statistics was conducted for all bird sampling data, as well as the data from the slaughterhouse gizzards, by using R. Due to human error, no bird samples were taken in trial 2. The gizzard evaluation data from trial 2 was not utilized further due to mixed-up samples at the slaughterhouse.

The observation of gizzard lesions were purely observational, thus, no statistic measurement was conducted on these data.

One-way ANOVA analysis was performed on relative gizzard weight, on sample 2 data (d 28 trial 1, and day 34 trial 3), by using R.

3.3 Experiment at the Ås gård

The experiment at the research facility at Ås Gård was performed in collaboration with five other master students, Aorihan, Biemujiafu Fuerjiafu, Ellen Cecilia Larsson, Huan Liu, and Sodbilig Wuryanghai. Thus, the material and method have been written in cooperation.

3.3.1 Animal housing

252 day-old male broiler chickens (Ross 308) were randomly placed into four equal sized pens (72cm x145cm). The floors were covered with a thick layer of wood shavings. The birds had access to both feed and water ad libitum. Room temperature the first week was approximately 28 ℃. 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 (approx. 30 ℃). Room temperature was reduced down to 22 ℃ over the three following weeks. At 5 days of age birds were moved from the pens to quail cages (d. 35cm x w.50cm x h.20cm); 4 birds from one pen were 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 gram were excluded from the experiment. The birds were exposed to continuous lightning since it was not possible to have complete darkness.

The quail cages were equipped with both a feeder and a water container, and trays underneath to collect excreta. The quail cages were organised 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.

3.3.2 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 of grit with whole-wheat, and particle flow were examined.

Diet and gritstone inclusion

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

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

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

Dissection and starvation

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

At day 20 feed was taken away at 21:00 and the birds were starved to 07:00 on day 21. On day 21, 1 bird was removed from each quail cage, marked with its cage number, and placed in a pen corresponding to its treatment with access to water and feed. The excreta trays were removed and excreta was collected. The birds had access to the feed for 5 hours, and the excreta trays were placed back after two hours of access to feed to collect excreta. The trays where left to collect excreta for the following 3 hours. After 5 hours, the bird was killed with a cranial blow followed by cervical dislocation and dissected. After dissection of all 48 birds, the birds in the pens were placed back into its respective quail cage and given access to feed and water. At day 21 feed was taken away at 21:00 and the birds were starved to 07:00 on day 22. On day 22, the birds were given access to the feed for only 30 minutes. Two birds from each treatment were killed with a cranial blow followed by a cervical dislocation, 60, 90, 120, 150, 180 and 210 minutes after commencement of feeding.

3.3.3 Laboratory work

All the samples were first thawed then homogenized, respectively.

Gizzard pH

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

Dry matter:

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

A representative sample was taken out, wet weight registered, and then dried in an oven at 103 ± 2°C overnight. The sample was placed in a desiccator until the sample was ambient before the dry weight was measured. Tare weight of crucible was subtracted from the gross weight of the sample to calculate net weight of the wet/dry sample (Equation 3).

After measured dry matter content of each digestive tract segment and faeces from day 21, intact whole wheat were picked out manually. To achieve this, the samples were diluted with water overnight. The whole wheat 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.

$$\frac{net\ weight\ of\ dry\ sample\ (g)}{net\ weight\ of\ wet\ sample\ (g)} \times 100\% = Dry\ matter\ (\%) \quad \textbf{(3)}$$

Apparent metabolizable energy (AME)

Apparent metabolizable energy (AME) of faeces from 13-18 and 18-21 days of age were performed by Frank Sundby. The AME was measured by subtracting the gross energy of excreta from the total gross energy of the feed consumed. To find gross energy a bomb calorimeter (PARR 6400 Bomb Calorimeter) was used.

Separation of gritstones from in the gizzard and faeces

Due to a relatively small amount gizzard content, the whole sample had to be used for dry matter determination. Thus, the particles had to be dissolved in water before using the floating method, as described in section 3.2.5.

The same process was used for faeces collected from 5-11 days of age. The faeces from each cage were homogenized. A 250 g sample was soaked in enough water to dissolve the particles. For faeces samples collected on 11-13, 13-18 and 18-21 days of age, the amount of gritstones 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 from 11-13, 13-18 and 18-21 days of ages were first homogenized and analysed for dry matter content. According to the Standard Wet Sieving Analysis Procedure from The Centre of Feed Technology/Fôrtek at NMBU (Miladinovic 2009), the samples should have been dried in the sieves for minimum 4 hours to determine the dry matter. However, due to practicalities and limited time, an alternative method was created to determine dry matter of the particle distribution.

100 grams of sample was dissolved in water for 10 minutes with the assistance of a magnet stirrer (IKA C MAG HS7) before wet sieved in a Retsch sieve shaker (AS 200 Control) with

amplitude 1.50 mm/g. Some additional water was used to rinse out the container with the sample to make sure all the particles were emptied into the sieves. Sieves size were 1.4 mm, 0.8 mm, 0.5 mm and 0.2 mm, and water pressure was at maximum. Sieving time was set to 2 min. with water, and 1 min. without water to shake off excess water. Each sieve was then weighed. From 4 replicas per treatment for all sample sets, a sample of approximately 2.5 g were taken out to determine the dry matter of respective particle size in the sieve. The average dry matter content was further used to calculate the particle distribution of the faeces on dry matter basis. To estimate a "wet tare sieve weight", empty sieves were shaken as mentioned and weighed. The average of 11 registrations was used when subtracting the tare weight from the gross registration of the wet sample. The content left in the sieves was washed out in a bowl and rinsed for gritstones, as described above. The gritstones were collected, and saved for further analysis.

Particle distribution of gritstones

Three representative samples from the original gritstones were dry sieved to find the actual particle size distribution of grit given to the birds. The tare of the sieve was first registered before about 100 grams of the initial gritstones were dry sieved for 1 minute on amplitude 1.00 mm/g on the Retsch sieve shaker (AS 200 Control), each sieve was then weighed and registered again before emptying the content of the sieves. All steps were repeated between each sample. Each type of gritstones was sieved with 4 replicates to get an average particle distribution. Similar procedure was conducted for gritstones that were found in the faeces and gizzard. Since the samples of gritstones from the gizzard content was small, the samples were pooled together from 12 replicas to 3 replicas. Only zeolite and quartz was detected in the gizzard content. The percentage particle distribution was calculated with the equation (1). Due to human error, the particle distribution was measured of the remaining gritstones in the bag, after the birds were fed. However, the particle distribution was assumed to be equal in the bag.

3.3.4 Statistical analysis

Statistical analysis was partly performed by Professor Birger Svihus using SAS software, except of grit content and grit/content ratio. This was calculated by student, using one-way ANOVA and Tukys' pair ways comparison in R.

4. Results and observations

4.1 Field trials

4.1.2 Field observations of grit consumption

Relatively few birds were eating at the time gritstones were given. The birds showed no immediate interest in the stones at the time grit was sprinkled on top of the feed.

The amount of gritstones residues was observed at day 8 for each trial, see figure 4 to 6. Litter particles and feed residues, in terms of dust particles, were observed together with the gritstones. Due to a large amount of residues in trial 1, a new observation was conducted on day 12, see figure 4(2).

For all trials, the feeders had gritstone residues at day 8. The highest amount of grit residue was observed for trial 1, where 9.5 g/bird was given. Less variation was observed between trial 2 and 3, where the amount of grit given was 7.5 g/bird and 6.9 g/bird, respectively. The grit residue for trial 1 at day 12, was similar to residues observed for trial 2 and 3 at day 9.

Figure 4: Amount of grit left in the feeders for trial 1. On average, similar amount was observed for the rest of the feeders. 1: Observation conducted on day 8. 2: Observation conducted on day 12. Photo: Osvald Østerhus

Figure 5: Grit residues for trial 2 on day 8. Approximately similar amount was observed for the rest of the feeders. Photo: Osvald Østerhus

Figure 6: Grit residues for trial 3 on day 8. Approximately similar amount was observed for the rest of the feeders. Photo: Osvald Østerhus

4.1.1 Particle distribution of diets

Particle distribution of each diet is shown in figure 7 to 9, respective to the period it was given. Particle distribution was similar between each trial for the starter and grower diet. For trial 1, the finisher diet deviated from the finisher diets in following trials with a higher content of larger particles in the range of 1.0 to 1.6 mm (26% versus approx. 16 %).

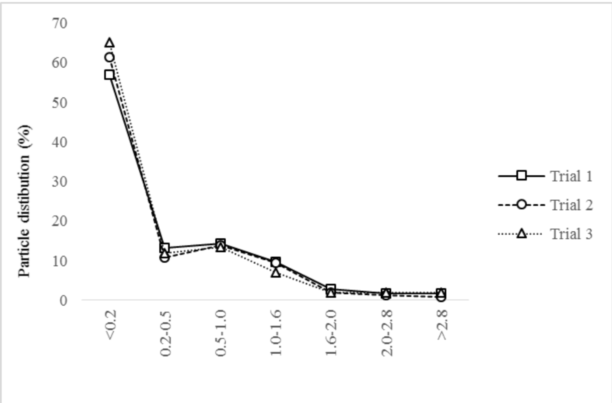

Figure 7: Particle distribution of starter diets, for each trial. Particle distribution was performed with the Standard Wet Sieving Analysis Procedure (Miladinovic 2009), and calculated as dry matter percentage.

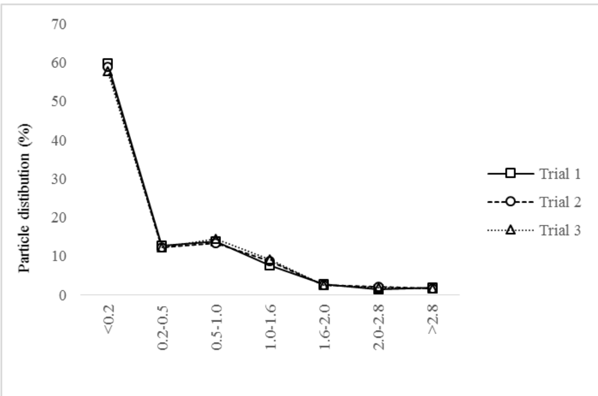

Figure 8: Particle distribution of grower diets for each trial. Particle distribution was performed with the Standard Wet Sieving Analysis Procedure (Miladinovic 2009), and calculated as dry matter percentage.

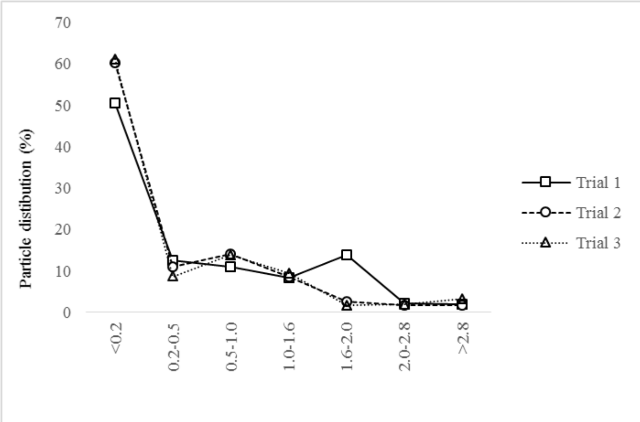

Figure 9: Particle distribution of finisher diets for each trial. Particle distribution was performed with the Standard Wet Sieving Analysis Procedure (Miladinovic 2009), and calculated as dry matter percentage.

4.1.3 Bird performance

For trial 1, the average bird weight for grit-fed birds at day 28 was 145 g lighter than of the control group, but the difference was not significant (P>0.05) (Table 4). A non-significant difference was also observed for trial 3, at day 33. Birds fed grit weighed on average 2182 g compared to 2003 g for birds fed without access to grit (P>0.05) (Table 5).

For all performance records at slaughter age, only minor differences were observed between the group given grit and the control group (Table 6). Recorded average slaughter weights for grit-fed birds in trial 1, 2 and 3 were 8 g, 35 g and 33 g larger than the control, respectively. For FCR and mortality, the recorded values were not consistent between trials.

For the performance data at the day of slaughter, there seemed to be an effect of trial period (Table 6). The recorded average slaughter weight for trial 3 was on average 95 g larger that of birds from trial 2. For FCR and mortality, trial 3 had the lowest values compared to trial 1 and 2 and showed less variation between treatments.

Table 4: Average live weight for birds sampled at day 28, trial 1 (n=10). Grit given 9.5 g/bird

Treatment	Grit	Control	P-value[1]
Mean	1565	1709	0.18
SD[2]	210.49	249.49	
SEM[3]	66.53	78.90	
Min.	1252	1410	
Max.	1959	2137	

[1]Significance level: P<0.05
[2]Standard deviation
[3]Standard error

Table 5: Average live weight for birds samples at day 33, trial 3 (n=10). Grit given 6.9 g/bird

Treatment	Grit	Control	P-value[1]
Mean	2182	2003	0.25
SD[2]	420.7	224.05	
SEM[3]	133.04	70.85	
Min.	1395	1573	
Max.	2768	2263	

[1]Significance level: P<0.05
[2]Standard deviation
[3]Standard error

Table 6: Summary of bird performance for trial 1, 2 and 3 at day of slaughter.

				Bird performance			
Trial[1]	Treatment	Age	Grit (g/bird)	BW[2] (g)	Daily gain (g/day)	FCR[3]	Mortality[4] (%)
1	Grit	32	9.5	1367	41.82	2.25	4.45
1	Control	32	-	1359	41.57	2.29	3.96
Diff.[5]				8	0.25	0.04	0.49
2	Grit	34	7.5	1464	42.21	2.36	3.33
2	Control	34	-	1429	41.18	2.32	2.91
Diff.				35	1.03	0.05	0.42
3	Grit	34	6.9	1558	44.98	2.17	2.59
3	Control	34	-	1525	44.01	2.18	2.62
Diff.				33	0.97	0.01	0.03

[1] Each trial was supplied with different diets. Identical diets were given within each trial.
[2] BW: Average slaughter weight.
[3] FCR: Feed conversion ratio (feed/gain (g/g)) adjusted for birds sampled during the trial periods, but not corrected for mortality.
[4] On-farm mortality. Values adjusted for the number of birds sampled during the respective trial period, but not for culls.
[5]Difference between grit fed birds and the control

4.1.4 Gizzard development and grit retention

Data from trial 2 is excluded from the following sections, thus when mentioning "both trials", I am referring to trial 1 and 3. Since the second sampling for trial 1 and 3 were conducted on different dates, referring to day 28 corresponds to the results from trial 1. Likewise, when discussing results from gizzards sampled at day 33, the result corresponds to trial 3.

Amount of gritstones retained in the gizzard

The average amount of grit retained in the gizzard was largest at day 9 for both trials (Table 7 & 8). The amount of grit was on average 1 to 3 g higher compared to gizzards sampled at an older age (Table 9 & 10). The average values obtained from the slaughterhouse gizzards were slightly higher than the values from samples taken at day 28 and 33 (Table 11 & 12). However, 50% of the gizzards had nil gritstones in their gizzard, and only 25% of the slaughterhouse gizzards had 0.23 g stones or more.

At day 9, the amount of gritstones in gizzards was observed to be 0.49 g for trial 1 (table 7) and 3.16 g in trial 3 (Table 8). However, a larger variation was detected between gizzards from trial 3. The maximum amount of grit observed in the gizzard at day 9 was found to be 6 g.

At day 28, the amount of grit content was on average 0.43 g with a maximum value of 2.27 g. For day 33, a slightly lower value was detected (0.33 g). The maximum value of grit retained was somewhat lower in trial 3 than for trial 1 (3.33g versus 4.21 g).

Similar to the results above, gizzards collected from the slaughterhouse showed a slightly lower average grit content in trial 1 compared to trial 3. However, the deviation from the mean was twice as large in trial 3, compared to the standard deviation in trial 1. Half of the gizzards from both trials contained nil gritstones. 75% of the observations were equal or below 0.19 g for trial 1 versus 0.27 g for trial 3. The highest level of gritstones were observed in trial 3, being as high as 19.5 g versus 10.2 g in trial 1.

The proportion of grit in the gizzards

At day 9, the highest grit-content ratio was observed for trial 3 (Table 8). At the highest levels, gritstones accounted for 75% of the weight of content in the gizzard. The average value was somewhat lower: 43%. Trial 1 had a considerably lower stone percentage than trial

3, the average being one fourth of the total content (Table 7). The highest proportion of grit in trial 1 was observed to be 47 %.

The stone percentage decreased at day 28 and 33 (Table 9 & 11). The largest decline was observed in trial 3, which reduced to 6.83 %. For trial 1, the grit percentage reduced to 8.45%. A similar trend was seen for the respective standard deviations.

Table 7: Body weight (BW), gizzard development (measured by empty gizzard weight) and grit present in the gizzard for grit-fed birds at day 9 for 1 (n=19*). Amount of grit: 9.5 g/bird

	BW [1] (g)	Gizzard[2] (g)	Gizzard, rel[3] (g/g)	Content (g)	Content, rel.[4] (g/g)	Grit[5] (g)	Grit/content[6] (%)
Mean	252	6.71	3.41	5.34	2.05	1.49	25.16
SD[7]	68.77	1.06	1.08	2.57	0.75	1.16	13.4
SEM[8]	15.78	0.24	0.25	0.59	0.17	0.27	3.07
Min.	114	4.91	2	1.25	0.83	0.15	3.34
Max.	336	8.71	5.94	9.24	3.57	3.64	46.54
Sum.[9]						28.38	

[1] Body weight/live weights of sampled birds
[2]Empty gizzard weight
[3]Relative gizzard weight (g empty gizzard,/g bodyweight)
[4]Relative gizzard content (full gizzard, g – /empty gizzard, g)/BW, g)
[5]Grit content in gizzard (g)
[6]Grit percentage of gizzard content
[7]Standard deviation
[8]Standard error
[9]Total amount of grit, sampled from all observations
*One sample was excluded due to unrealistic value for bird weight (BW 742 g).

Table 8: Body weight, gizzard development (measured by empty gizzard weight) and grit present in the gizzard for grit-fed birds at day 9 for trial 3 (n=20) Amount of grit: 6.9 g/bird

	BW [1] (g)	Gizzard[2] (g)	Gizzard, rel[3] (g/g)	Content (g)	Content, rel.[4] (g/g)	Grit[5] (g)	Grit/content[6] (%)
Mean	280	8.23	2.93	6.64	2.37	3.16	42.81
SD[7]	21.92	1.08	0.31	2.27	0.81	1.67	18.12
SEM[8]	4.9	0.24	0.07	0.50	0.18	0.37	4.05
Min.	243	6.42	2.31	0	0	0.8	0
Max.	323	10.36	3.79	10.25	3.80	6.05	75.29
Sum.[9]						63.32	

[1] Body weight/live weights of sampled birds
[2]Empty gizzard weight
[3]Relative gizzard weight (g empty gizzard,/g bodyweight)
[4]Relative gizzard content (full gizzard, g – /empty gizzard, g)/BW, g)
[5]Grit content in gizzard (g)
[6]Grit percentage of gizzard content
[7]Standard deviation
[8]Standard error
[9]Total amount of grit, sampled from all observations.

Table 9: Gizzard development (measured by empty gizzard weight) and grit present in the gizzard at day 28 (n=10) for trial 1.

	Gizzard (g)			Gizzard, rel.[1] (g)			Content (g)			Content, rel.[2] (g/g)			Grit[3] (g)	Grit/content[4] (%)
Treatment	Grit	Control	P-value	Grit	Control	P-value	Grit	Control	P-value	Grit	Control	P-value	Grit	Grit
Mean	17.24	17.56	0.827	1.11	1.03	0.345	4.75	6.53	0.31	0.31	0.39	0.403	0.43	8.45
SD[5]	3.16	3.29		0.22	0.16		4.13	3.43		0.26	0.22		0.83	11.96
SEM[6]	1.00	1.04		0.07	0.05		1.31	1.09		0.08	0.07		0.26	3.78
Min.	13.62	12.52		0.82	0.86		0.76	0.17		0.04	0.01		0	0
Max.	21.79	22.84		1.52	1.26		10.63	10.78		0.61	0.76		2.74	34.04
Sum.[7]													4.27	

[1]Relative gizzard weight (g empty gizzard,/g bodyweight)
[2]Realative content weight (g content/g bodyweight)
[3]Grit content in gizzard (g)
[4]Grit percentage of gizzard content
[5]Standard deviation
[6]Standard error
[7]Total amount of grit, sampled from all observations.
[*]Significance level: $P<0.05$

Table 10: Gizzard development (measured by empty gizzard weight) and grit present in the gizzard at day 34 (n=10) for trial 3.

	Gizzard (g)			Gizzard, rel.[2] (g/g)			Content, (g)			Content, rel.[3] (g/g)			Grit[4] (g)	Grit/content (%)
Treatment	Grit	Control	P-value	Grit	Control	P-value	Grit	Control	P-value	Grit	Control	P-value	Grit	Grit
Mean	19.96	19.48	0.813	0.91	0.98	0.377	7.97	5.83	0.452	0.36	0.29	0.613	0.33	6.83
SD[5]	4.72	4.19		0.10	0.21		7.28	4.92		0.36	0.25		0.34	8.54
SEM[6]	1.49	1.33		0.03	0.07		2.30	1.56		0.11	0.08		0.11	2.7
Min.	12.10	15.40		0.71	0.70		1.56	0		0.09	0		0	0
Max.				1.06	1.44		21.24	13.65		1.18	0.68		0.94	27.73
Sum.[7]													3.33	

[1] Body weight/live weights of sampled birds
[2]Relative gizzard weight (g empty gizzard,/g bodyweight*100)
[3]Realative content weight (g content/g bodyweight*100)
[4]Grit content in gizzard (g)
[5]Standard deviation
[6]Standard error
[7]Total amount of grit, sampled from all observations.
[*]Significance level: P<0.05

Table 11: Gizzard development (measured by empty gizzard weight) and grit retention for gizzards collected at the slaughterhouse, n=100 (trial 1).

	Gizzard (g)			Grit[1] (g)
Treatment	Grit	Control[2]	P-value	Grit
Mean	21.10	19.62	0.00832*	0.69
SD[3]	3.46	3.42		1.73
SEM[4]	0.35	0.44		0.21
Min.	15.34	13.29		0
1st Qu[5]	18.82	17.42		0
2st Qu	20.50	19.62		0
3st Qu	23.34	21.00		0.19
Max.	31.29	29.53		10.12
Sum.[6]				48.55

[1]Grit content in gizzard from 70 birds (two values excluded due to an error in measurement).
[2]n=60 due to exclusion of badly cut gizzards from the slaughter house
[3]Standard deviation
[4]Standard error
[5]Qu = quantiles
[6]Total amount of grit, sampled from all observations (n=100)
* Highly significant (significance level: $P<0.05$)

Table 12: Gizzard development (measured by empty gizzard weight) and grit retention for gizzards collected at the slaughterhouse, n=102 (trial 3)

	Gizzard (g)			Grit[1] (g)
Treatment	Grit	Control[3]	P-value	Grit
Mean	21.11	18.82	1.07e-05*	0.85
SD[4]	4.2	2.96		2.62
SEM[5]	0.42	0.29		0.31
Min.	14.31	13.04		0
1st Qu[6]	18.48	16.6		0
2st Qu	20.33	18.35		0
3st Qu	23.24	20.73		0.265
Max.	35.44	26.09		19.43
Sum.[7]				60.87

[1]Grit content in gizzard from 72 birds
[4]Standard deviation
[5]Standard error
[6]Qu = quantiles
[7]Total amount of grit, sampled from all observations
*Extremely significant (significance level: $P<0.05$)

Influence of grit on gizzard weight

The amount of grit present in the gizzard had no influence on the empty gizzard weight (see Appendix 1). Correcting for the weight of the bird, there seemed to be a slight tendency for an increased gizzard weight with an increasing amount of gritstones in the gizzard at day 28, (figure 12). However, no such relationship could be observed for relative gizzard weight at day 33 (figure 13). For the respective gizzards, as previously mentioned no significant effect of grit on gizzard weight could be detected in both trials (Table 9 & 10).

Contrary to these results, the gizzards collected at the slaughterhouse showed a highly significant effect of grit on gizzard weight, for both trials (Table 11 & 12).

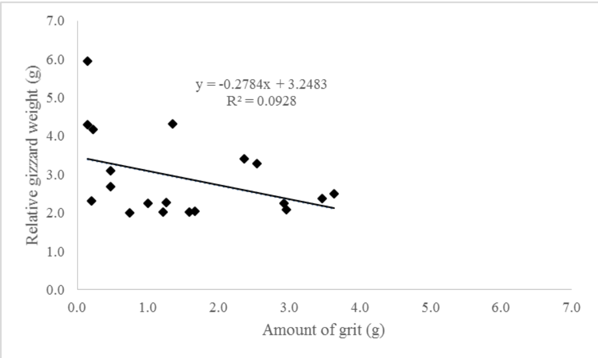

Figure 10: Linear regression of relative gizzard weight (gizzard weight/bird weight) versus amount of stones present (g) at day 9, trial 1 (n=19). *One sample were excluded due to unrealistic value for bird weight (BW 742 g).

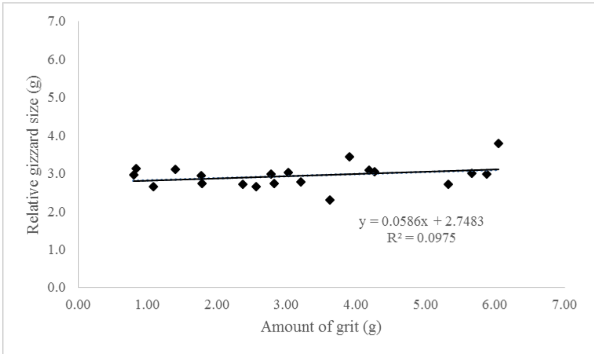

Figure 11: Linear regression of relative gizzard weight (gizzard weight/bird weight) versus amount of stones present (g) at day 9, trial 3 (n=20).

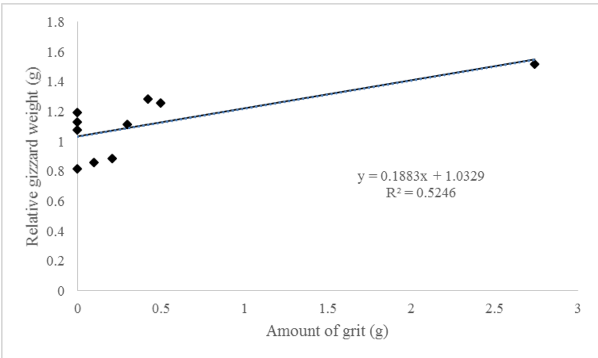

Figure 12: Linear regression of relative gizzard weight (gizzard weight/bird weight) versus amount of stones present (g) at day 28, trial 1 (n=10).

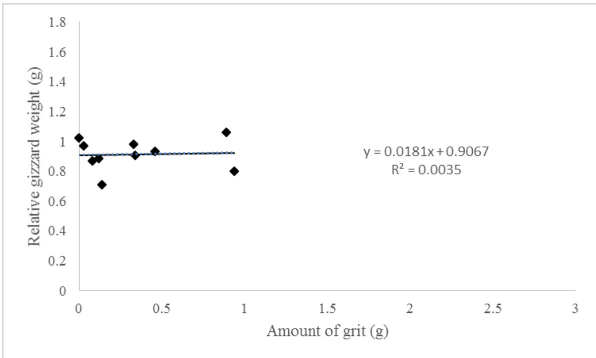

Figure 13: Linear regression of relative gizzard weight (gizzard weight/bird weight) versus amount of stones present (g) at day 34, trial 3 (n=10).

Influence of grit on the holding capacity of the gizzard

The holding capacity of gizzard seemed to increase simultaneously with an increase in gizzard content, at day 9 for trial 1 (figure 14). This was not observed for trial 3 (figure 15), nor for birds at day 28 and 34 (figure 16 & 17). For birds on these days, no significant effect on content was seen between grit-fed birds and birds without access to grit (Table 9 & 10).

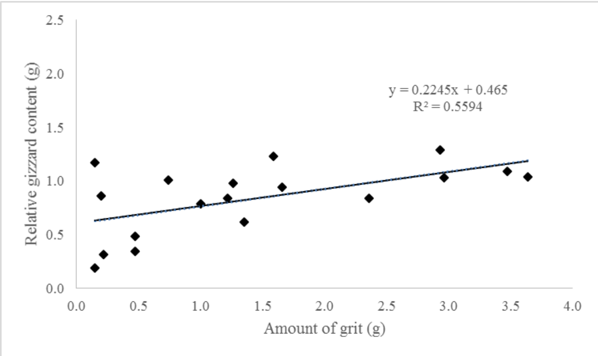

Figure 14: Linear relationship between the relative amount of gizzard content (g) and amount of gritstones (g) in the gizzard at day 9, trial 1 (n=19)

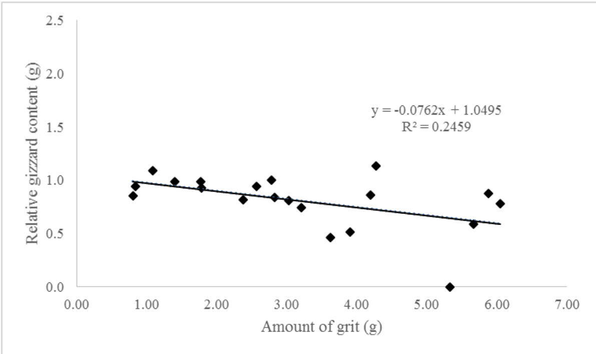

Figure 15: Linear relationship between the relative amount of gizzard content (g) and the amount of gritstones (g) in the gizzard at day 9, trial 3 (n=20).

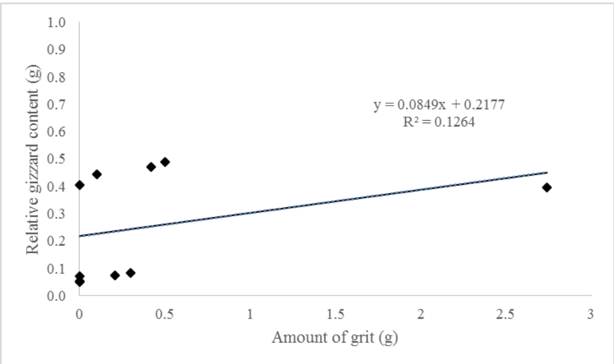

Figure 16: Linear relationship between the relative amount of gizzard content (g) and amount of gritstones (g) in the gizzard at day 28, trial 1 (n=10)

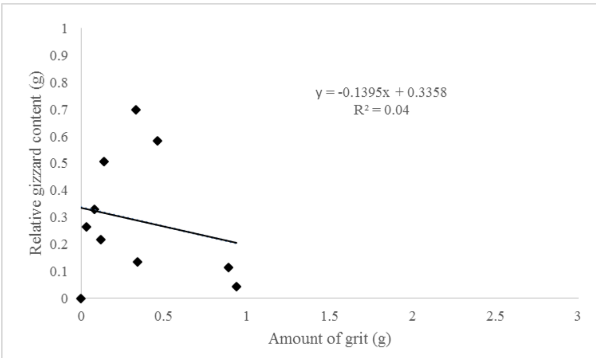

Figure 17: Linear relationship between the relative amount of gizzard content (g) and amount of gritstones (g) in the gizzard at day 34, trial 3 (n=10)

4.1.5 Gizzard lesions

Gizzard lesions were observed already at day 9 (figure 18) The majority of gizzards had a moderate to severe score (trial 1: 20/20; trial 3: 13/20). Overview of gizzard lesions from sampling days 28 and 33 is shown in figure 19.

For gizzards collected at the slaughterhouse, no clear trend was observed for severity of lesions and use of gritstones, as shown in figure 20. Gizzard from the grit fed group had a somewhat higher prevalence of moderate lesions score in trial 1, but they had a lower ratio of severely scored gizzards, than the control group. Trial period seemed to have an influence on gizzard score, trial 1 having a lower ratio of moderately scored gizzard than trial 3.

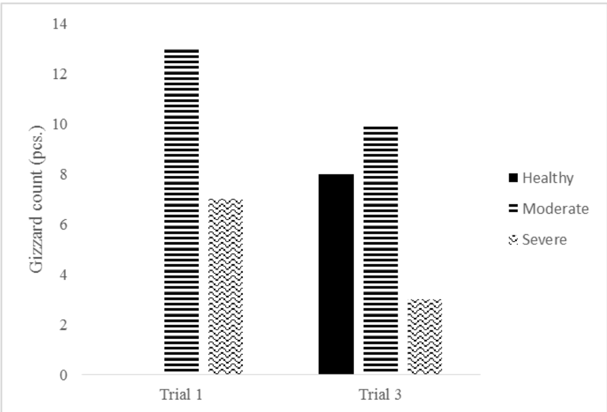

Figure 18: Gizzard score count (pcs.) for grit fed birds, sampled day 9. n = 20

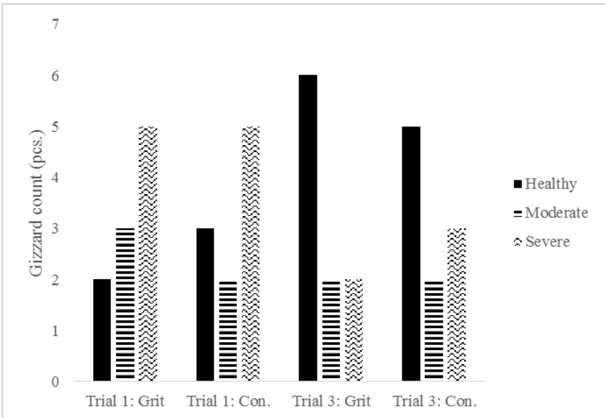

Figure 19: Gizzard score count (pcs.) for grit fed and control birds at day 29. *Trial 3: Samples were taken at d 33. n = 10

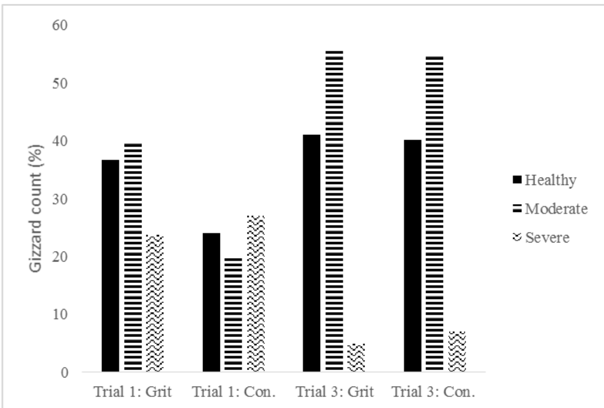

Figure 20: Gizzard score count (%) from gizzard collected at the slaughterhouse, for birds given grit (Grit) or no grit (Con.) for trial 1 and 3. Gizzard were scored as healthy, moderate or severe lesions. Trial 1 Grit n=100, Control n=71. Trial 3: n=102

4.1.6 Particle distribution of gritstones

Initial particle size is presented in figure 21. The ordered particle dimension was 2-3.5 mm. Sieving the stones showed that 78% of the particles were above 2 mm. Only 2 % of the stones were 3.55 mm or larger. The majority of the stones were in the range of 2.00 mm to 2.5 mm, 44% respectively.

Figures 22, 23 and 24 compare the initial particle distribution to the grit found in the gizzards at different sampling days, for the three trials, respectively. For trial 1, a higher proportion of larger stone particles were found at all sampling days, compared to the initial grit. Trial 1 had approximately 14% higher amount of stones equal to and larger than 2.8 mm, compared to the initial grit given to the birds.

Particle distribution procedure was also conducted on trial 2 (figure 23). In spite of the fact that gizzards were mixed at the slaughterhouse, it was assumed that gizzards with detectable gritstones, belonged to birds fed grit. No clear difference was detected in the particle size between the grit found in the gizzard and the initial grit. The same trend as shown in trial 1 were observed for trial 3 (figure 24) and was particularly evident in grit samples found on day 33, where 55% of the stones were above or equal to 2.8 mm.

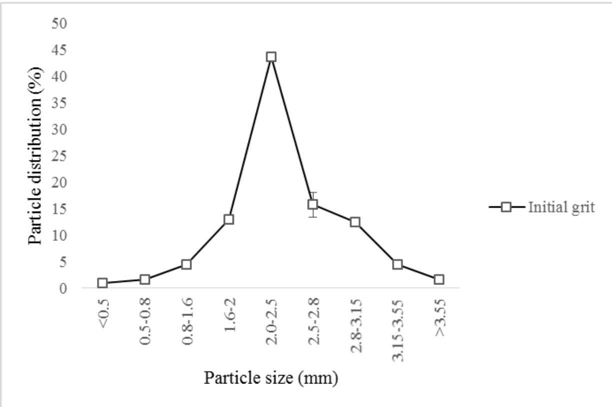

Figure 21: Particle distribution of initial grit (as fed)

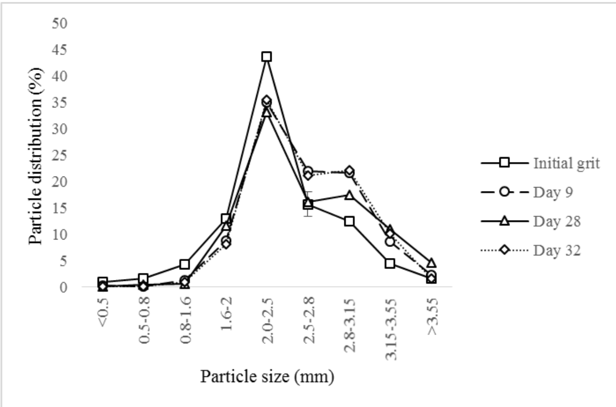

Figure 22: Particle distribution of grit in trial 1. Graph showing the distribution of initial grit (as fed), and of grit found in the gizzard at day 9 (n=20), 28 (n=10) and 32 (n=100). Mean±SEM is given for initial grit.

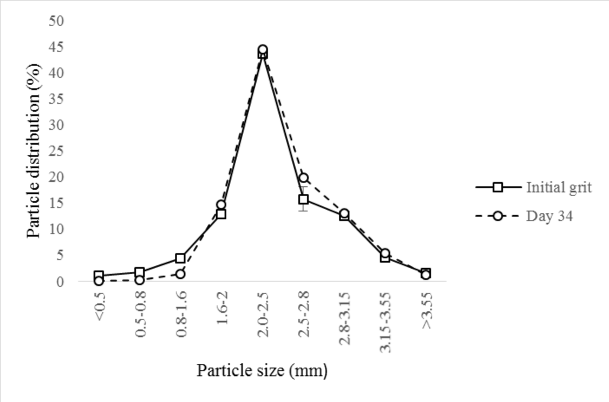

Figure 23: Particle distribution of grit in trial 2. Graph showing the distribution of initial grit (as fed) and grit found in the gizzards at day 34 (n=102). Mean±SEM is given for initial grit.

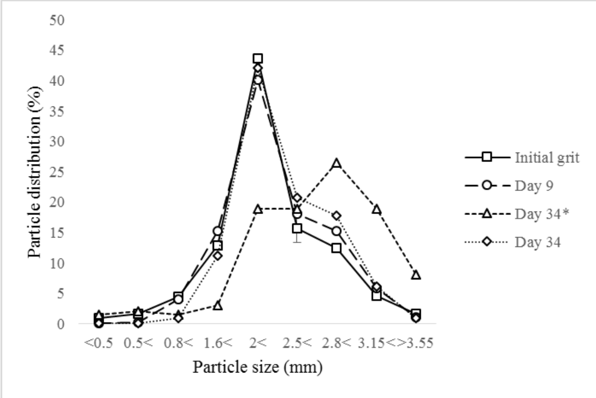

Figure 24: Particle distribution of grit in trial 3. Graph showing the distribution of initial grit (as fed), and of grit found in the gizzard at day 9(n=20), 34*(n=10) and day 34 (n=102). Mean±SEM is given for initial grit.

4.2 Experiment at Ås gård

Results reported herein will be a part of presented results in the master thesis from the following students: Aorihan, Biemujiafu Fuerjiafu, Ellen Cecilia Larsson, Huan Liu, and Sodbilig Wuryanghai. Interactions between whole wheat and gritstones will be discussed in detail by Aorihan "Effect of feeding diet with whole wheat with and with out grit stones on performance and digestive characteristic in broiler chickens" and will only briefly be mentioned herein. Particle flow is not within the scope of this thesis, and will be discussed in the thesis of Biemujiafu Fuerjiafu "The effect of the gritstone on feed passage rate".

4.2.1 Grit consumption

Birds fed quartz and zeolite grit consumed on average 9.3 g of stones per bird (figure 25). Birds given marble consumed considerable fewer stones, with an average of 5.8 g.

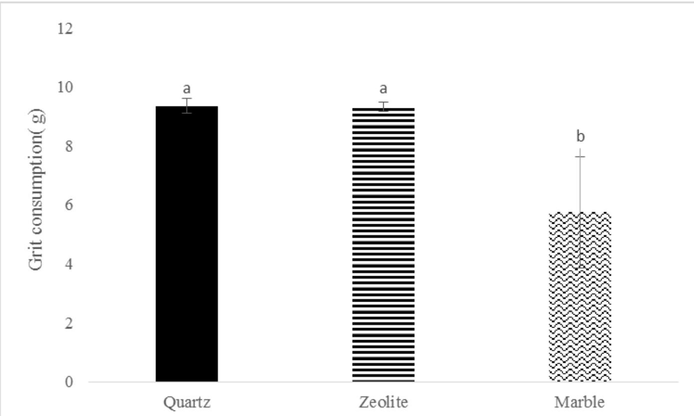

Figure 25: Average grit consumption for three different types of gritstones; quartz, zeolite and marble. ±SD. Grit given 9.5 g/bird. Means with different letters are significantly different. (P<0.05). (n=48)

4.2.2 Bird performance

Feed intake was not significantly different for birds fed without grit and those with access to insoluble stones for all periods, including 18-21 days of age where birds got the diet consisted of 15% whole wheat, see Table 13. For marble fed birds, a significant reduction in feed intake was observed for all periods, compared to above mentioned treatments, with the exception of age interval 11-13 and 18-21 days of age. Herein, the feed intake was similar to the both the quartz fed birds and the control.

Zeolite-fed birds showed an increased weight gain between 11 to 21 days, compared to the control and the marble-fed birds, but weight gain was not significantly different from the quartz-fed birds. FCR was not significantly different between birds fed gritstones or birds fed without grit, see Table 13.

Table 13: Summary of bird performance for different gritstones, control, quartz, zeolite or marble, at various age intervals. Diets given; 0-11 d: starter diet, 11-18 d: grower diet, 18-22 d: Mixed diet; 15% whole wheat and 85% starter diet. Number of birds: 5-13 days of age n=48, 18-21 days of age n=36

Age (d)	Control	Quartz	Zeolite	Marble	Sq. MSE	P-value[1]
Feed intake (g)						
5-11	275a	271a	269a	252b	10.2	<.0001
11-13	161ab	165ab	170a	157b	9.9	0.0174
13-18	495a	496a	516a	467b	26.9	0.0009
18-21	270ab	283a	281a	246b	29.6	0.0137
5-21	1201a	1215a	1235a	1121b	53.2	<.0001
11-21	926a	944a	966a	870b	49.4	0.0002
5-18	931a	932a	955a	876b	37.0	<.0001
Weight gain (g)						
5-11	225a	225a	219a	203b	15.8	0.0044
11-13	139	145	145	138	10.7	0.2535
13-18	384ab	384ab	406a	361b	24.7	0.0009
18-21	135	143	152	124	28.2	0.0956
5-21	882a	893a	921a	825b	47.6	0.0001
11-21	657bc	672ab	702a	622c	43.3	0.0006
5-18	748a	754a	769a	702b	35.6	0.0003
Bird weight (g)						
13	495	518	497	504	31.5	0.2953
18	898	894	898	838	73.0	0.1315
21	1043	1070	1107	996	104.7	0.0823
FCR (g/g)						
5-11	1.23	1.21	1.23	1.24	0.053	0.4888
11-13	1.16	1.14	1.18	1.14	0.057	0.3641
13-18	1.29	1.29	1.27	1.30	0.038	0.4497
18-21	2.05	2.07	1.88	2.10	0.409	0.5578
5-21	1.36	1.36	1.34	1.36	0.040	0.6490
11-21	1.41	1.41	1.38	1.40	0.049	0.4023
5-18	1.25	1.24	1.24	1.25	0.028	0.7811

[1]Significanse level: P<0.05

[a,b] Means with different letters are significantly different.

4.2.3 Gizzard development and grit retained in the gizzard

Differences in empty gizzard weight were only detected at day 13, where both quartz and zeolite fed birds had a significantly heavier gizzard than birds with access to marble, table 14. For marble, empty gizzard weights were not significantly different, compared to the control. Differences in relative empty gizzard weight were not observed for any gritstone type.

The amount of content in the gizzard was significantly higher for birds fed quartz grit than birds for birds fed zeolite or marble, but not compared to the control. No differences were detected between the zeolite, marble and the control. At day 21, the marble group had lower content weight than the other treatments. For relative gizzard content, a significant value was only observed at day 13, where birds fed with zeolite or marble had a significantly lower amount of content in the gizzard than the quartz fed birds.

A larger amount of grit was retained in the gizzard of quartz fed birds than both marble and zeolite birds. Similarly, the grit/content ratio was higher for the quartz fed group, except for day 13 where no significant difference could be observed between quartz and zeolite.

Quartz grit seemed to be retained in the gizzard for longer, compared to the zeolite and marble grit (Table 14) where a larger amount of grit were observed in the faeces from 11 to 13 day of age for the zeolite and marble.

4.2.4 The effect of grit on gizzard function

The effect of different grit types on gizzard function was measured by AME (MJ/kg), particle distribution in faeces, in addition to pH level in the gizzard content.

Gritstones did not affect AME for birds fed grower pelleted diet or starter diet supplemented with whole wheat, see table 15.

From day 11 to 13 days of age, there was an effect of gritstones on the particle size in faeces, where birds fed quartz grit had a significantly lower amount of particles bellow 0.2 mm, than both the control and marble fed birds (figure 26). Birds fed marble had the largest amount of particles below 0.2 mm but the difference was not significantly different compared to the control. Zeolite feed birds had a higher amount of particles in the range of 0.5 to 0.2 mm, than the other treatments. From day 13 to 18 days of age, no significant differences were observed for any gritstone type (figure 27).

When whole wheat was included in the diet, birds fed with zeolite grit had a higher amount of particles in the range of 0.5 to 0.2 mm, compared to birds fed with quartz, marble or birds without grit (figure 28). Birds fed quartz grit had a higher amount of particles in the range 1.4 to 0.8 mm.

Gritstones did not affect the pH level in the gizzard in grower feed, nor when whole wheat was included as for day 21, shown in Table 16.

Table 14: Summary of data for gizzard development, measured by empty and holding capacity (gizzard content) for different gritstones, control, quartz, zeolite or marble, at different age intervals. Diets given; 0-11 d: starter diet, 11-18 d: grower diet, 18-22 d: Mixed diet; 15% whole wheat and 85% starter diet. Number of birds: 5-13 days of age n=48, 18-21 days of age n=36

Age (d)	Control	Quartz	Zeolite	Marble	Sq. MSE	P-value[1]
Gizzard (g)[2]						
13	10.5b	11.7a	11.0ab	10.3b	0.97	0.0051
18	14.0	13.8	14.0	13.4	2.11	0.8723
21	16.5	16.7	16.8	15.3	1.90	0.1853
Relative gizzard[3] weight (g/g)						
13	2.14	2.26	2.21	2.05	0.226	0.1356
18	1.56	1.55	1.58	1.61	0.256	0.9313
21	1.59	1.56	1.52	1.54	0.172	0.8105
Content (g)						
13	6.6ab	8.5a	6.1b	5.7b	1.96	0.0076
18	8.1	8.1	7.1	8.1	5.11	0.9559
21	8.6ab	10.2a	9.6ab	7.2b	2.51	0.0296
Relative gizzard[4] content (g/g)						
13	1.34ab	1.63a	1.24b	1.14b	0.394	0.0230
18	0.89	0.91	0.79	0.97	0.559	0.8879
21	0.82	0.94	0.86	0.73	0.212	0.1156
Grit in gizzard (g)						
13	-	3.13a	0.86b	0.00c	0.58	<.0001
18	-	1.64a	0.09b	0.00b	0.94	<.0001
21	-	3.31a	0.53b	0.00b	0.96	<.0001
Grit/content (g/g)[5]						
13	-	0.35a	0.12a	0c	0.07	<.0001
18	-	0.17a	0.01b	0b	0.23	<.0001
21	-	0.30a	0.06b	0b	0.08	<.0001
Grit passage (g/g)[6]						
5-11	-	0.45a	0.39a	0.26b	0.085	<.0001
11-13	-	0.12b	0.18a	0.20a	0.062	0.0065
13-18	-	0.10a	0.07b	0.00c	0.042	<.0001

[1]Significanse level: P<0.05
[2]Empty gizzard weight
[3]Relative gizzard weight (g empty gizzard,/g bodyweight)
[4]Relative gizzard content (full gizzard, g – /empty gizzard, g)/BW, g)
[5]Grit content in gizzard (g)
[6]Grit percentage of gizzard content
[a,b] Means with different letters are significantly different.

Table 15: Apparent metabolizable energy (AME) MJ/kg in faeces for different gritstones, control, quartz, zeolite or marble, at different age intervals. Diets given starter diet, 11-18 d: grower diet, 18-22 d: Mixed diet; 15% whole wheat and 85% starter diet. Number of birds: 5-13 days of age n=48, 18-21 days of age n=36

AME (MJ/kg)	Control	Quartz	Zeolite	Marble	Sq. MSE	P-value[1]
Age (d)						
13-18	13.5	13.7	13.6	13.6	0.40	0.776
18-21	14.0	14.2	14.1	13.5	1.04	0.350

[1]Significanse level: P<0.05
[a,b] Means with different letters are significantly different.

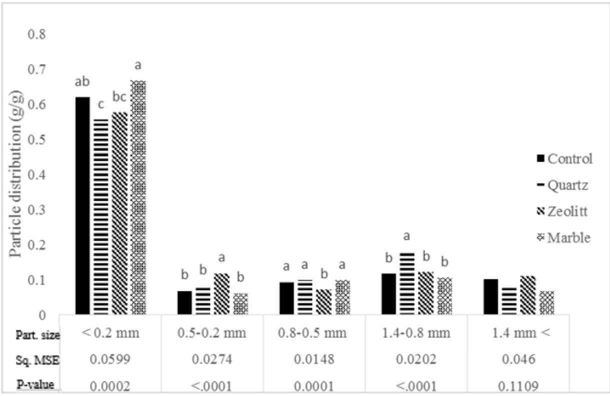

Figure 26: Particle distribution of faeces form 11-13 d for different types of grit versus the control. Means with different letters are significantly different. Particles size with no letters are not significantly different. Birds fed starter diet.

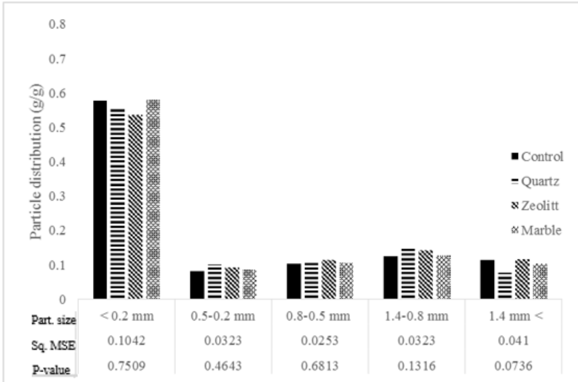

Figure 27: Particle distribution of faeces from 13-18 d for different types of grit versus the control. There were no significant differences between treatments and the control. Birds were given grower diet.

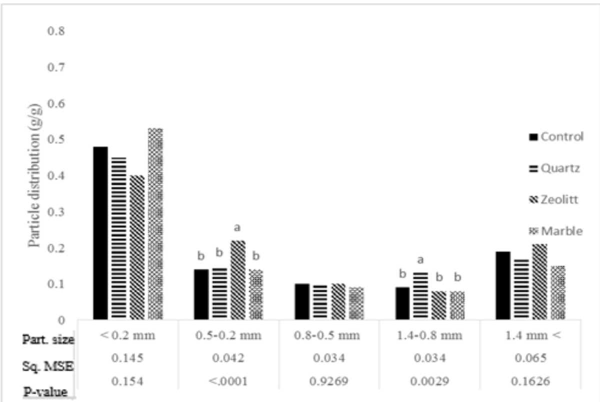

Figure 28: Particle distribution of faeces from 18-21 d for different types of grit versus the control. Means with different letters are significantly different. For particles size with no letters the treatments were not significantly different. Birds were given 85% starter diet mixed with 15% whole wheat

Table 16: pH level for different gritstones, control, quartz, zeolite or marble, at different age intervals. Diets given; 0-11 d: starter diet, 11-18 d: grower diet, 18-22 d: Mixed diet; 15% whole wheat and 85% starter diet. Number of birds: 5-13 days of age n=48, 18-21 days of age n=36

Age (d)	Control	Quartz	Zeolite	Marble	Sq. MSE	P-value[1]
pH						
13	3.1	3.4	3.5	3.5	0.59	0.3280
18	3.3	3.6	3.6	3.1	0.80	0.3888
21	3.0	2.9	2.8	2.8	0.48	0.6643

[1]Significanse level: P<0.05
[a,b] Means with different letters are significantly different.

5. Discussion

5.1 Bird performance

The combined results of the field trials and the experiment at the research facility did not provide clear evidence of beneficial effects on the birds' performance, nor for any adverse effects, by giving the birds access to insoluble gritstones. A slight improvement of 0.5% to 3% increase in average slaughter weights was observed for birds fed quartz grit, compared to the birds fed without grit for all field trials. Although, such a difference in individual bird weights may seem to be minor, it may be of economic value for the producer, if consistent. Considering a flock of 25 800 birds, a difference in 30 g will account for 0.7 tons of meat.

Lack of replications precludes statistical analysis of recorded performance data for the field trials. However, when this was done for birds collected pre-slaughter, the few birds sampled were not able to demonstrate a significant effect on bird weight, thus indicates that gritstones do not have a convincing effect on bird weight. Neither observations under controlled conditions confirmed a beneficial effect of grit supplementation on bird weight. This is in agreement with similar observations of Riedel (1950); Rowland and Hooge (1980); Spencer and Jenkins (1963). Scott and Heuser (1957) on the other hand, did provide significant evidence for a higher weight gain by supplementing the chicks with insoluble grit. Similarly, Balloun and Phillips (1956) found a considerable increase in weight gain by supplying the birds with gritstones. The authors reported a 12 % increase in weight gain for birds fed granite grit compared to those without access to grit. However, in their second experiment, the authors did not manage to reproduce these results. Birds fed grit showed a non-significant advantage in weight gain compared to the control. Still, the authors considered the numerical difference to be large and concluded that birds with access to grit showed a strong tendency for a higher weight gain, compared to birds without access to grit. Inconsistency in results between studies may be due to differences in gritstone characteristics and experimental design.

For our experiment, FCR was not markedly affected by feeding insoluble grit, which was also observed by Bennett et al. (2002); Bennett and Classen (2003); Itani (2015); McIntosh et al. (1962); Rowland and Hooge (1980). A similar feed intake was observed between birds fed insoluble grit and birds fed without grit. This is surprising since the physical restraints and limited space in the gizzard should, in theory, constrain the amount of feed particles eaten. As Moore (1998a) pointed out the gritstone ingested by the birds is a trade-off between the space for nutritious elements and gritstones. At an early age, gritstones accounted for almost half of the content weight in the gizzard. The observation indicates that birds fed grit somehow compensate for the quantity of stones present in the gizzard, being able to consume as much feed as birds without access to grit. Similar conclusions were drawn by Itani (2015). A possible explanation is that the gizzard muscles do become more developed by extra grinding activity, and thereby increases its holding capacity (Itani 2016, pers. comm., 12. April). However, this was not seen in our study. Also, Moore (1998b) emphasized that the increase in holding capacity in the gizzard is not necessarily beneficial for the grinding process. Another possible explanation could be that the bird either uses the crop to a larger extent or spend a longer time feeding, so the amount of feed processed in the gizzard are the same but more evenly distributed through the day.

Spencer and Jenkins (1963) on the other hand, observed a significant reduction in feed consumption for birds given insoluble grit compared to the control group. Despite this effect, live weights were not significantly different. Thus, indicating that grit fed birds are more efficient in utilizing the feed material. Unfortunately, no statistical comparison was performed on the data due to concerns regarding if the values were normally distributed. However, both Balloun and Phillips (1956) and Tepper et al. (1939) also found improved feed efficiency for birds given access to gritstones, compared to those fed without grit. The increased feed efficiency may have resulted from a more developed gizzard for grit fed birds, than for birds without grit. The effect of grit on gizzard development will be discussed in detail in section: 5.2.

The majority of papers examining the effects of different types of grit concerning their chemical composition have mainly been focusing on soluble versus insoluble stones. In our experiment feeding soluble grit did not influence the broiler chickens performance in terms of total weight or feed efficiency. However, marble fed birds did show a significantly lower weight gain and feed intake, which explains the non-excitant difference in FCR between marble-fed bird, insoluble grit-fed birds and the birds fed without grit. As already discussed in the literature section, soluble stones will contribute to the birds' mineral requirement, and may thus disturb the birds' mineral balance. Particularly in the case of calcium rich stones adverse effects on chicks' performance have been observed (Heuser & Norris 1946). This suggests that the birds are not able to regulate grit consumption according to their maximum mineral requirement. According to Scott and Heuser (1957), the bird's requirement of hard grit like substances will outweigh the bird's ability to control the calcium intake, and as a result, they consume a substantially higher level of calcium than needed. Although, the authors did not observe lower bird weight for calcite fed birds compared to the control. Layers do have a higher requirement for calcium than broilers, due to the vast amount of calcium needed in eggshell formation. Thus, it is likely that adverse effect is not present or less pronounced in layers. However when formulating diets for young layer chicks it may have to be taken into consideration, as Heuser and Norris (1946) observed for young White Leghorn cockerels.

No clear difference in performance was found between the two types of insoluble grit, although a numerical tendency to higher weight gain was observed for zeolite fed birds. Difference in birds' preferences for type of gritstone was not examined in this study. However, a higher grit consumption was observed for birds fed quartz and zeolite grit compared to marble and likely due to the calcium effect discussed above. According to Scott and Heuser (1957), chicks seemed to show a preference for different types of insoluble grit, comparing feldspar towards granite grit. No differences were observed in bird performance between the two grit types. This is not surprising, considering that granite does in fact mainly consist of feldspar (approx. 70% feldspar, minimum 20 % quartz and 1-10% other minerals) (Oftedahl 1980).

Although a slight difference was observed for mortality between birds fed grit and non-grit birds, the two trials appeared to have a somewhat higher in value in mortality for grit-fed birds as opposed to birds fed without grit. Inconsistency between trials implies that there is no obvious effect of grit supplementation on mortality. Neither have any publications reported mortality effects on feeding insoluble grit, which is not surprising, considered that the ancestral birds did consume gritstones, and many of the wild species today still process this habit of ingesting stones. Naturally, if gritstones had been causing a higher mortality the trait of ingesting stones would have diminished, seen in an evolutionary perspective. However, a higher mortality has been observed for birds force fed with calcite grit (Heuser & Norris 1946), likely due disturbance in the bird's mineral balance. The authors did not find such an effect for birds given granite grit.

5.2 Gizzard development

For the field trials, differences in gizzard weight and relative gizzard weight were not detectable for grit-fed birds versus the control. Despite this observation, gizzards from the grit-fed birds, collected at the slaughterhouse showed a significantly higher gizzard weight compared to those from the control ($P<0.01$). This is peculiar, but may be due to too few observations for the birds sampled pre-slaughter. That said, since gizzard weight does not merely illustrate the effect of gritstones, but includes the size of the bird, care must be taken when considering the relevance of these data. Under controlled conditions, access to either insoluble or soluble grit did not influence the gizzard weight, except of quartz grit at an early age. Correspondingly, relative gizzard weights did not differ at any age. A tendency to a higher body weight at an early age for birds fed quartz grit do explain the higher gizzard weight at the respective age. An increase in bird size will naturally increase the organ size, at least to a certain degree. Generally speaking, structural components has been shown to increase the size and holding capacity on the gizzard (reviewed by Svihus 2011). In the studies reviewed by Svihus (2011), the source of structure has mainly been whole grains, oat hulls and litter particles, like wood shavings. As already mentioned in the literature section, selective retention of these particles will force the gizzard muscles to work for longer to reduce the particles size, and as a result, the gizzard muscles will become larger and more developed. Not surprisingly, although contrary to our results, similar observations have been seen in birds fed grit as opposed to birds without access to gritstones (Gionfriddo & Best 1999). Several authors have found a significant relationship between gizzard development and grit supplementation (Garipoglu et al. 2006; Heuser & Norris 1946; Itani 2015; Spencer & Jenkins 1963). In contrast, Jones and Taylor (1999) and Bethke and Kennard (1926) did not find any changes in the gizzard morphology by giving the birds access to gritstones.

No positive relationship for gizzard weight and quantity of grit retained in the gizzard was found at any age. Correcting for the bird weight a slight tendency was noticeable for gizzards sampled a few days pre-slaughter, for trial 1. However, this was not done for stone content, thus the stone quantity in the gizzard might have been indirectly influenced by bird size. A larger bird may have a larger stone consumption and a larger quantity of grit retained in the gizzard, than a smaller sized bird. Different from our results, Itani (2015) found a slight correlation at an early age, but for gizzards sampled at the end of the experimental period, no such relationship was detected. Itani (2015) suggested that the lower grit percentage in the gizzard at the end of the experiment resulted in a less grinding activity, thus reduced the stimulation effect on the gizzard. The fact that less grinding is needed, may in turn cause the gizzard size to shrink due to a cost-benefit relationship for excess capacity of the gizzard organ (Starck 1999). For example, in wild bird species, where seasonal changes forces the birds to change diets, reversible alterations of the gizzard size are well known to occur, accordingly to the diet composition (reviewed by Klasing 2005). Other authors, for instance Spencer and Jenkins (1963) found a strong correlation between gizzard weight and quantity of stones retained in the gizzard ($P<0.001$). Contrary to our results, as mentioned in the paragraph above, both Itani (2015) and Spencer and Jenkins (1963) found a significant effect of grit on gizzard weight compared to the control.

Gritstones did not convincingly affect the holding capacity of the gizzard. However, several studies have reported an increase not only in the gizzard mass but also for the holding capacity of the gizzard (reviewed by Svihus 2011). The importance of increased holding capacity in the gizzard may not be beneficial for the grinding efficiency, as emphasized by Moore (1998b) but may still have a positive influence on the birds' digestion due to a potentially larger amount of feed particles will be retained for longer in the gizzard, thus being exposed for preliminary for a prolonged time. The holding capacity in the studies reviewed by Svihus (2011) was measured by the amount of gizzard content in gram, and may thus be less suited for measuring holding capacity in grit-fed bird versus control due to different particle density of stones compared to organic material (ie. feed particles and litter). Nevertheless, due to practicalities the particular method was utilized in this study. Even so, gritstones did not increase the gizzards' holding capacity, compared to the control. Quartz grit did seem to increase gizzard content compared to the zeolite stones. However, this was most likely due to a substantially higher content of quartz grit retained in the gizzard, as opposed to zeolite stones. Similar to these data no clear relationship was observed between increasing relative content weight and the amount of gritstones. Only a slight tendency was observed for field trail 1 at day 9. Considering that the total gizzard content does include gritstones, implies that the two variables are not independent of each other, thus the reliability of these models can be questioned.

5.3 Digestion

Supplementation of gritstones showed no effect on AME for birds fed pelleted diets, with or without whole wheat inclusion. In contrast, several authors have found significant effects on

ME/AME (Evans et al. 2005; McIntosh et al. 1962; Smith & MacIntyre 1959) and digestibility (Smith & MacIntyre 1959; Smith 1960) by grit supplementation. A well-developed gizzard muscle is generally considered to improve the functionality and efficiency of the gizzard organ, thus enhancing the birds' digestion and increase energy utilization (Svihus 2011). In our study, no convincingly difference was detected between birds fed grit and birds fed without access to grit, which might explain the non-existent effect on AME. However, Bethke and Kennard (1926); Itani (2015); Scott and Heuser (1957) have not been able to confirm the necessity of enlargement in gizzard size for bird performance or improvements in energy utilization. In fact, in the study of Itani (2015) showed that, despite a significant difference in gizzard size between birds fed grit and birds without grit, the birds performed equally well. Hetland et al. (2003) observed an increased gizzard size for several types of structural components, including gritstones. However, the effect on digestion varied between the different sources of structure. For instance, gritstones did neither affect the feed efficiency, nor starch digestibility but significantly reduced the duodenal particle size ($P<0.05$). Out hulls on the other hand, did improve the feed efficiency and starch digestibility but showed no influence on particle size of the duodenal content, whereas only ingested wood shavings did improve concentration of bile acid in the gizzard content. Hetland et al. (2003) concluded that the gritstones do aid the grinding process, although not necessarily through stimulation of the gizzard muscle, but by sheer forces created between the surface area of feed material and the stones, as pointed out by Moore (1998b). One possible explanation for lack of effect on digestibility and feed efficiency for gritstones in both Hetland et al. (2003) and our study might be the inhibition of the translational movement, caused by the quantity of gritstones present in the gizzard. Consequently, then limiting the gizzards ability to mix the feed material and digestive juices, thus exposing less material to preliminary proteolysis. Another possible explanation is that the increase in small particles belongs to the degradation process of NSPs (oat hulls and wood shavings), and does not improve the energetic value for the bird, despite the increased surface area for digestive enzymes.

Our results show that feeding grit does not necessarily reduce particle size in faeces. In fact, quartz grit resulted in a higher proportion of lager particles compared to birds fed without grit. No difference was observed in particle distribution at a later age for the grower diet. As mentioned above, Hetland et al. (2003) did observe a higher amount of small particles for birds given grit. However, the difference between the two studies is most likely due to different ways of sampling. In our study, the collection of quantitative samples of faeces were conducted for a given time interval, while Hetland et al. (2003) collected duodenum samples at the day of slaughter. The relevance of this observation is clear because Hetland et al. (2003) samples were taken prior to jejunum, the absorption site. A higher absorption of small particles will increase the proportion of potentially larger indigestible particles in faeces. In addition and most importantly, not all gritstones are being retained in the gizzard, but passes through the intestines and is eliminated in the faeces, also observed by Itani (2015). The same author noticed only a few stones in the intestines at day of dissection. Hetland et al. (2003) did not report such an observation. In our study, the amount of stones found in the faeces from 11 to 18 days of age, was originally thought to be too few to influence the particle size of the faeces considerably, thus the data was not adjusted for stone weight. However, the amount of insoluble grit in faeces may also explain our observations. Samples collected for age interval 11 to 13 days, the amount of quartz grit in faeces might explain the significant higher percentage of particles in the range of 1.4 to 0.8 mm, consequently reducing the particles size below 0.2 mm.

Although, the amount of zeolite in faeces was significantly higher compared to quartz grit, this trend was not observed for zeolite. This may be explained by the stones characteristics of zeolite. Zeolite has hardness equal to calcific gritstones (“3” on Mohs scale of hardness). Quartz on the other hand, has a value of 7 on Mohs scale. This implies that wear forces in the gizzard will affect zeolite size to a larger degree, than quartz grit. This could in turn wear down zeolite stones unequally, thereby explaining the non-significant relationship of percentage level for small zeolite particles in faeces. Interestingly, faeces from the marble group showed a significant higher amount of small particles (<0.2 mm) from 11 to 13 days of age, compared to both zeolite and quartz. Considering that marble grit is categorized as a soluble grit type and is measured to a hardness of 3-4 on Mohs scale, this observation may be explained by a rapid degradation of the marble grit in the gizzard into small, almost non-detectable stone particles (<0.2 mm). This might explain the higher percentage of small particles, and in combination of the reduced percentage of small particles for zeolite and quartz grit, might explain the significant difference between insoluble and soluble gritstones for particles below 0.2 mm. If faeces was corrected for stone weight, it is logic to assume a similar response as seen for the following period (13-18 d), with less or no significant effect of grit on particle size.

Particles distribution of faeces from 18 to 21 days of age, clearly demonstrate an increased proportion of large particles (1.4 mm<), obviously caused by the whole-wheat inclusion in the diet. Grit was refed on day 18, 19 and day 20, and since the likelihood of grit appearing in the faeces increased, the particle size was adjusted accordingly. There seemed to be an effect of quartz grit, showing a larger particle size (0.8 to 1.4 mm) despite the fact that the amount of grit in faeces is accounted for. As mentioned previously the effect of particle reduction may show the opposite trend for faeces compared to prior the absorption site in the small intestine. The effect of zeolite grit is harder to explain, with reduced particle size between 0.2 to 0.5 mm. It may be that zeolite grit is less effective in grinding, than quartz due to the hardness characteristic of the stone.

5.4 Gizzard pH

Gritstones did not influence the pH level in the gizzard in a pelleted grower diet, nor when whole wheat was supplemented the starter diet, as for day 21. A possible explanation may be the lack of a grit effect on the gizzard development. A larger, more developed gizzard will be better able to retain material in the gizzard, as mentioned earlier. Thereby increasing the secretion of gastric juices, followed by a reduction in pH.

In literature, there has been little focus on the effect of gritstones on pH in the digestive tract of the bird. Farner (1943) is one of the few who has reported an effect of gritstones. The author observed an increase in pH for birds fed diets with 4 to 8 % inclusion of limestone grit. This is not surprising since limestone is pure calcium carbonate, a well-known alkaline mineral with the ability to neutralize acids, thus increasing the pH level. For the same reason, pH in digesta for laying hens are reported to be higher than for broilers, due to the high calcium level in the diet (reviewed by Svihus 2011). Surprisingly, this was not evident from our result, where pH in digesta for marble fed birds was not significantly different from that of digesta from birds fed insoluble stones, or those fed without gritstones. In Farner (1943) study gritstones were mixed in the diet, whereas in our study it was sprinkled on top of the feed. It may be that birds in Farner (1960) study were not able to regulate their gritstone consumption accordingly to their calcium need. Spencer and Jenkins (1963) reported that birds fed with a fixed level of grit mixed in the feed had a higher grit consumption than birds fed ad libitum, but where the gritstones were fed in a separate trough. In our study, marble fed bird showed a substantially lower gritstone consumption, being almost half the amount of stones compared to birds fed quartz and zeolite. Another possible explanation could be the amount of grit retained in the gizzard at the time of measurements. For marble, no grit was detectable in the gizzard for any of the dissection days. Considering the low gritstone intake and the fact that grit were removed on day 13, one can assume that the grit stones were already dissolved and let out the gizzard, thus not influencing the pH substantially.

5.5 Gizzard lesions

Gritstones had no obvious beneficial effect on prevalence of gizzard lesions. This is contrary to the observations of Almquist (1938a) who observed a reduction in both frequency and severity of gizzard lesions by giving the birds access to grit. A possible explanation may be the degree of gizzard development. Almquist (1938a) did observe larger and firmer gizzard muscle for birds fed grit and grit like substances, than those birds fed without these components. As mentioned in the literature section, the author did not find any effect on gizzard lesions when using very finely ground grit. However, the size used was far below the range used in our experiment (<0.180 mm). It is likely that gritstones beneath certain size will no longer stimulate the gizzard efficiently. Similarly, the retention time and access to grit in our study may have not been sufficient to stimulate the gizzard for a longer period, thereby being insufficient in limiting the prevalence of gizzard lesions.

Bird et al. (1937) stressed the fact that gritstones may have different effects of various types of lesions, and that grit may have an adverse effect on crater erosions if the lesions are already present at hatch. As observed in our experiment, severe lesions were already present in gizzards from birds sampled at 9 days of age. Although the adverse effect was not observed by Almquist (1938a), it indicate that further focus should be directing in preventing gizzard lesions, in terms of crater lesions at hatch.   Adverse effects on gizzard linings has been observed with use of very fine grit.

Tepper et al. (1939) observed extensive damage to the inner lining of the digestive tract of birds fed granite waste. It may be that other interactions between small particles, to dust size, and the gastrointestinal lining will occur.

One of predisposing factors that been linked to gizzard lesions is *Clostridium perfringens*. (Gjevre et al. 2013). A reduction in *Clostridium perfringens* counts has been observed for diets with structural components, like whole wheat (Bjerrum et al. 2005). Similar to several publications (reviewed by Svihus 2011), Bjerrum et al. (2005) observed an increase in gizzard size for birds receiving whole wheat as opposed to birds fed pellet diets. The indirect effects of gizzard development, for instance by enhancing the absorption, thus decreasing optimal growth conditions for *Clostridium perfringens*, could in turn be preventive for gizzard lesions. This could explain the beneficial effects of gritstones on gizzard lesions as observed by Bird et al. (1937) and Almquist (1938a).

6. Summaries and conclusions

There was neither convincingly beneficial nor adverse effects on broiler chicken performance and digestion, by supplementing insoluble grit to pelleted diets with or without whole-wheat inclusion.

Lacking effect on performance and digestion is consistent with the lack of improvement in gizzard development. However, it seems that insoluble stones do aid in particle reduction to a certain degree, most evident when whole wheat was supplemented the diet.

Use of calcific stones should be used with care and should not be recommended in use for broiler chickens, since it may influence and disturb the birds' mineral balance, thereby reduce bird performance.

Today, Norwegian slaughterhouses will not face challenges by retained grit in gizzard at slaughter age. The fact, that no adverse effects were detected by feeding insoluble grit may point to that there is still some benefits to gain from using gritstones for broilers. Feeding gritstones may stimulate birds' normal behaviour in terms of seeking and pecking behaviour, which is important from a welfare perspective and considered more and more important by the consumers. In addition, with the withdrawal of antibiotics and consumers demand for a coccidiostats free production, the importance to combine different strategies is important, to strengthened the birds' robustness.

Future research should look further into the effect of optimal feeding of grit, optimal grit size for different ages and possibilities of different types of insoluble types of gritstones.

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8. Appendix

Linear regression of empty gizzard weight and amount of stones retained in the gizzard

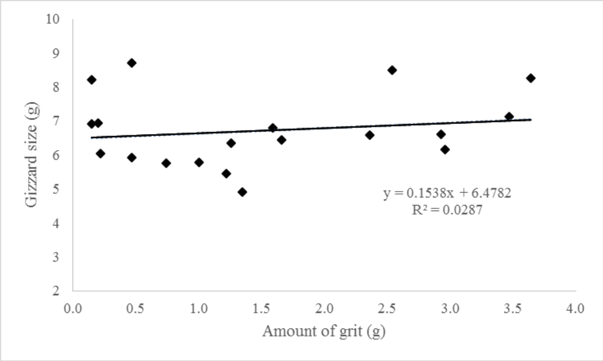

Figure 1: Linear regression of empty gizzard weight and amount of stones present (g) at day 9, trial 1 (n=19). *One sample were excluded from the dataset due to unrealistic value for bird weight (BW 742 g).

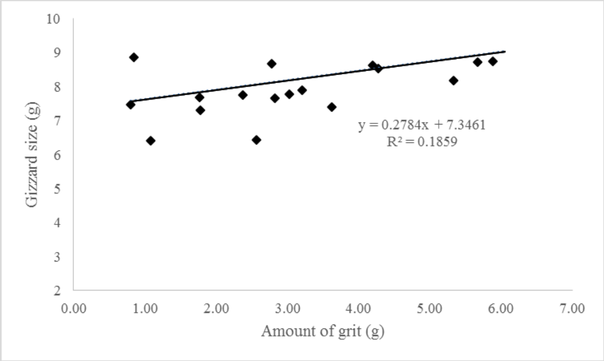

Figure 2: Linear regression of empty gizzard weight and versus amount of stones present (g) at day 9, trial 3 (n=20).

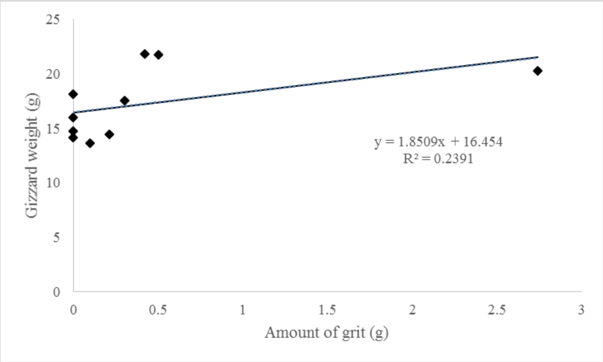

Figure 3: Linear regression of empty gizzard weight and amount of stones present (g) at day 28, trial 1 (n=10).

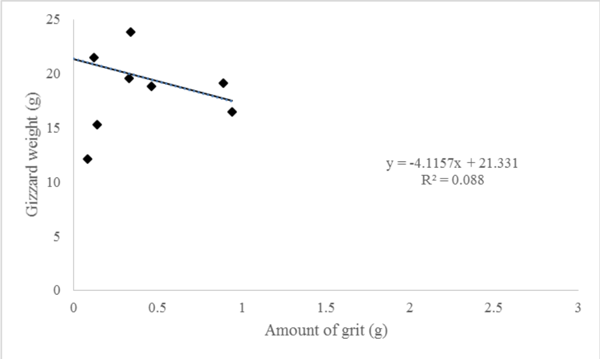

Figure 4: Linear regression of gizzard weight and amount of stones present (g) at day 34, trial 3 (n=10).

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