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Eating Patterns of Broiler Chickens Fed Insoluble Grit and its Effect on Intake Variation, Retention Time, Performance and Gizzard Development.

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ABSTRACT

In a field trial, the current study tested the effects of insoluble granite grit stones supplemented to broiler chickens on growth performance and gizzard stimulation. Particle size distribution and variation in the amount of grit retained in the gizzards were also examined at different age intervals. 880-day-old broiler chicks were allocated randomly and equally to 11 floor pens and maintained on a commercial pelleted diet. The trial consisted of two treatments: a control, or no-grit group (NGG), and a grit treatment group (GG). During the first three days, all pens were managed under the same conditions. On day four, chicks in the GG were given grit stones (sieved to a size range of 3.55 to 1.6mm) on separate plates. Grit provision was initiated with 150 grams per pen, and continued on days seven, nine and eleven respectively with 350g per pen, making 1200g in total or 15g per bird. On day 18, uneaten grit was withdrawn from all pens. At 10, 21 and 28 days, randomly selected grit-fed birds were killed and frozen for laboratory analysis. At 30 days of age, 208 gizzards from both groups (416 in total) were collected from the slaughter house. 72 gizzards from the 208 were randomly selected to examine the variation in the amount of grit retained at the end of the trial. Finally, grit from the rest of the gizzards was pooled in one sample and analyzed for particle distribution. The findings showed grit supplementation did not impair nor improve ($P > 0.05$) broiler overall performance compared to the NGG. Grit significantly increased ($P < 0.001$) gizzards' size, and their full and empty weight in the grit-fed birds. Birds from the GG showed individuality in the amount of grit ingested at different ages and the variation in the amount retained increased with age. Grit seemed to be evenly distributed among gizzard contents, which provided more contact surface with the feed and efficient particles grinding. On average, grit accounted for almost 36% of the total gizzard content weight at 10 and 21 days, but dropped to 21% at 28 days of age. Retained grit varied from zero to 368 particles. Of the 208 gizzards, 8% retained nil, and 3% contained very few, whereas the remainder retained a considerable amount even 12 days after withdrawal. The average number of stones found in the gizzards was 161, 231 and 140 stones, while the maximum amounts were 243, 366 and 368 stones at 10, 21 and 28 days respectively. It was observed in the small intestine that some grit of different sizes were passing through the gizzard almost completely intact during various growth periods. Gizzard grit appeared to have smoother surfaces with rounded corners due to erosion as opposed to the rough, irregular and sharp edges of the initial grit. Particle size distribution showed a conspicuous reduction in larger particle proportion and a subsequent increase in the medium and small particles fraction.

Key words: Grit Stones, Gizzard Stimulation, Grit Retention, Intake Variation, Particle Size.

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1. Introduction

Poultry diets must be well balanced in terms of nutritional elements, so that birds are not over or under-fed, and to achieve the best possible outcome. If feed ration is inadequate in terms of critical components, chicks or mature birds will exhibit signs of nutritional deficiency which inevitably leads to a slower growth rate, a drop in egg production, and overall poor performance (Sullivan & Gleaves 1977; Konashi et al. 2000; Burgos et al. 2006). Coupled with nutritionally sound feed, the bird must be healthy and able to extract nutrients efficiently through digestion. Therefore, a well-functioning digestive system is critical for superior performance ie. growth rate, FCR (feed conversion ration), feed intake and egg production.

As digestion starts with mastication or chewing, food must be well ground and softened. This would increase the surface area of the digesta as Goodband et al. (2002) described, and reasonably allow for more efficient enzymatic breakdown. However, the avian digestive tract is sharply distinguished from that of other monogastric animals like pigs: it differs in some significant aspects (McDonald 2002). Chicken lack teeth for mastication, but rather, they are equipped with a well-developed muscle 'the gizzard' that grinds the food into a finely divided state, acting like a grinding mill (Akester 1986). Besides its grinding function, Hetland et al. (2002) concluded that an active gizzard plays a role as a mixing compartment for digestive juices and nutrients, which is favorable for digestion. Although grinding food or coarse food particles is the most conspicuous role, improving digestibility (Hetland & Svihus 2001; Hetland et al. 2002; Svihus et al. 2004a), regulating digesta flow (Svihus et al. 1997; Svihus 2011a; Tesfamariam et al. 2013), inhibiting microbial population (Gabriel et al. 2003; Bjerrum et al. 2005; Santos et al. 2008; Singh et al. 2014), and preventing proventriculus dilatation (Riddell 1976; Taylor & Jones 2001) are also ascribed to a developed and well stimulated gizzard.

Considering its prominent value, a substantial number of reports and reviews have been published to elucidate the importance of the avian gizzard, examining its distinctive characteristics and its role as a vital organ for adequate and enhanced digestion (Gabella 1985; Akester 1986; Ferket 2000; Svihus 2011a; Svihus 2011b; Svihus 2012).

Furthermore, the impact of feed form, (Choi et al. 1986; Abdollahi et al. 2014), particle size (Amerah et al. 2008; Auttawong 2012), whole cereal addition (Svihus et al. 1997; Hetland et al. 2002; Wu & Ravindran 2004; Svihus et al. 2004a) and structural component inclusion (Hetland et al. 2005; Hetland & Svihus 2007; Sacranie et al. 2012) on poultry gizzard stimulation and development have been investigated. The stimulation is also exhibited phenotypically by a more pronounced shape, harder wall and increased size. Svihus (2011a) attributed this enlargement "to the stimulative effect of the increased grinding activity on the size of the two pairs of gizzard muscles".

Previous studies have confirmed that birds ingest hard structural material to promote the mechanical processing of food within their gizzard. For instance, McIlhenny (1932) stated that "in order to digest the hard woody roots that constitute their food, geese require a large powerful gizzard and a lot of grit". McIlhenny (1932) added, in the case where there is insufficient sand or gravel, birds must travel long distances where grit is abundant and get their requirements. Similarly, Scott and Heuser (1957) indicated that the grinding process was aided significantly when birds received hard insoluble grit that helped in the abrasion of the coarse particle of food. Duke (1986) found 10% improved digestibility when grit was added to birds' diet. In 1927, Mangold cited in Wings (2007) also reported a 25 to 30% increase in digestibility of chickens feed following grit addition. Although grit may not be necessary for survival, many birds require it as a source of nutrients in addition to its mechanical function (Gionfriddo & Best 1999)

For the aforementioned potential benefits, grit stones could be used to promote grinding efficiency of the gizzard, as a cheap source of minerals, and as a stimulating component if high uniformity in intake amongst birds is found essential. However, due to grit intake variation, it is possible to find either that some birds over-consumed grit, or that other birds ingested only few. The residence time of stones in the gizzard and their dynamics in the intestinal tract also require investigation. Accordingly, a field trial was carried out to study and understand these aspects in addition to the effect of grit on utilizing pelleted feed. This enables a better assessment of the efficacy of grit, quantity offered, time of withdrawal, effect of intake variation and the retention in the gizzard at different ages.

2. Literature Review

2.1. Digestive System and Digestion:

In order to ensure that feed is being converted efficiently into nutrients consistently throughout a bird's life, it is of utmost importance to have a thorough understanding of the general digestion mechanism in poultry. Therefore, knowledge of the avian digestive system and organs involved is essential. As mentioned previously, feed processing is a prerequisite for nutrients absorption.

As the bird eats, feed is swallowed and stored temporarily in the crop, where no enzymatic or absorptive reactions are present. Although it is considered a transitory storage pouch, manipulating the feeding system can alter crop functionality. For example, Svihus (2014) postulated that intermittent feeding resulted in a larger feed quantity stored in the crop and retained for a longer period of time. This allowed for further softening of feed particles by additional moisture contact, lowered pH due to fermentation and influenced exogenous enzyme activity.

Feed then slides down to the first part of the stomach (glandular portion) or the proventriculus where chemical digestion initiates. In this compartment, the compound sub-mucosal glands secrete pepsinogen, a precursor for the enzyme pepsin (Thomson 1969), while hydrochloric acid (HCl) is produced by highly specialized acid-secreting gland cells (Dibner & Richards 2004). Conversely, earlier observation by Toner (1963) detected no difference between the two types of glands. The author confirmed that acid and proteolytic enzymes are secreted solely by the sub-mucosal gland cells. Nonetheless, (Dibner & Richards 2004) suggested that there is no storage function associated with the bird’s glandular stomach. This is consistent with Rynsburger (2009) findings, who inferred that the residence time of digesta in the proventriculus is very short and similarly its contact with the secreted juices. To compensate for this insufficient contact time, a thorough mixing of water, HCl and pepsin is facilitated by the muscular movements in the second part of the stomach or the ventriculus, as digesta is released (Svihus 2014).

As the gizzard blends juices and nutrients, a concomitant interruption of the digesta flow by refluxes in the opposite direction (within different compartments) in the digestive tract was reported (Klasing 1999). For instance, a digesta reflux between the gizzard and proventriculus (and vice versa) causes a prolonged enzymatic and HCl contact with large particles which is favorable for both chemical and mechanical breakdown. Besides mixing, the ventriculus (gizzard) which is made up of strong myelinated muscles with a koilin layer, crushes and pulverizes feed particle in two ways (Svihus 2014). Due to the rhythmic contraction of the gizzard's muscles, feed particles are firstly crushed between the koilin plaques as Dzuik and Duke (1972) cited in (Akester 1986) outlined, and secondly, because of the rubbing against other particles that adds a more abrasive action (Svihus 2014). Grit stones for example, act jointly with gizzard contraction to crush and grind the edges of the coarser particles (Klasing 1999).

Finally, after being reduced to an appropriate size, particulates flow to the small intestine where digestive fluids are also added from the pancreas and the liver simultaneously for further digestion. The small intestine's epithelium is covered by millions of microscopic projections or villi that provide a higher surface area for enzyme secretion and eventually most of the nutrients' absorption (Dibner & Richards 2004).

1.1. Importance and Ways of Gizzard Stimulation

The significance of a well-developed gizzard has been evidenced in a large number of publications. In addition, ways of stimulating the gizzard were also highlighted in an ample number of studies.

The effect of mash versus pelleted feed on broiler performance was examined by (Amerah et al. 2007). Their data showed that birds receiving mash feed had a relatively larger gizzard and higher apparent metabolisable energy (AME) compared to the pellet group. These results suggested that pelleting did not stimulate gizzard due to insufficient grinding activity and thus reduced its function to that of transit. However, due to a higher feed intake in the pellet group, birds were heavier and had a better feed to gain ratio. Similarly, Abdollahi et al. (2014) tested the effect of mash, steam conditioned pellets and reground pellets in a sorghum based diet on broiler performance and digestibility.

Feeding pellets resulted in shorter relative length of small intestine and caeca compared to mash diet where an increase in gizzards' weight was also seen. Low proportion of structural components like coarse particles in pelleted diet did not stimulate the gizzard since there was no necessity for additional grinding activity. As a result, pellet-fed birds had smaller gizzards with lower efficiency in nutrients utilization. Accordingly, starch, protein and fat AME was higher for the mash fed birds.

Moreover, the influence of particle size on bird performance was also investigated. Amerah et al. (2008) concluded that coarser grinding improved feed efficiency in both corn and wheat based diets. This was justified by an increased gizzard activity coupled with a longer residence time which in turn enhanced digestion. (Bjerrum et al. 2005) suggested that feeding whole wheat for broiler chicken not only promoted the gizzard's mechanical stimulation but allowed it to act as a disease prevention organ. This was associated with a longer content retention time and a subsequent increased HCl production in the proventriculus. The resulting lower pH therefore restricted bacterial proliferation and reduced the number of salmonella.

Sacranie et al. (2012) reported that birds with access to coarse hulls developed the heaviest gizzard compared to the birds deprived of hulls. The large particle size of the coarse hulls and their hardness explained this observation. The large hull particles do not pass through until ground to a certain critical size (Hetland et al. 2002). Accordingly, a longer retention time means an increased muscular activity to meet the greater demand for grinding and hence a better AME was observed.

Riddell (1976) studied the influence of fiber in the diet on dilation of the proventriculus (Hypertrophy) in chicken. A hypertrophy occurs when the proventriculus has a greatly enlarged thin wall and no stark demarcation with the gizzard. It is a secondary response triggered by a finely ground diet, and a poor gizzard development. A dilated proventriculus can cause significant carcass contamination when its thin wall is ruptured at processing (Crespo & Shivaprasad 2003). Riddell (1976) indicated that birds fed the control ration had smaller gizzards and 37% of them had a proventriculus that was as large as the gizzard or larger. In contrast, birds fed the ration containing oat hulls had a proventriculus and gizzard that were normal in size and conspicuously demarcated.

1.2. Grit Definition:

Gionfriddo and Best (1999) defined grit as "stones and rock fragments ingested by birds, excluding very fine particles such as dust or ash". Mackie (2002) also described grit as sand particles, stones or pebbles used by different categories of birds to enhance the grinding process of food in their highly specialized, muscular gizzard. According to Adeniji (2009) grit can be classified into soluble and insoluble particles. Soluble grit includes limestone source and oyster shell, which when dissolved in the low pH of the ventriculus can supply the bird with calcium and other minerals. Insoluble grit includes silica, mica and other indigestible stones that are comparatively harder and more resistant to the acidic environment, hence they are retained for a longer period of time .

1.3. The Importance of Grit for Avian Species:

The value of grit for avian species has been recognized and studied for decades (Waite 1935; McCann 1939; Balloun & Phillips 1956; Harper 1963; Trost 1981; Bennett & Classen 2003; Evans et al. 2005). In particular, (Gionfriddo & Best 1999) noted that the interest in studying grit use has been motivated by its perceived and numerous advantages seen in domesticated fowl and wildlife species. Gionfriddo and Best (1999) reviewed extensively the use of grit, its function and significance for different bird species. The authors reported most of the commonly attributed roles of ingested grit: it facilitates the mechanical breakdown of food in the gizzard, supplements the diet with essential minerals, and may have other plausible digestive activities (mixing food with enzymes, stimulating digestive organs).

Studying the characteristics of grit used by wild birds enables a better understanding of their proneness to ingesting grit-like pesticides or toxic material, thus offering an effective way to control and limit one of the most common causes of bird mortality (Sánchez-Bayo 2012). Another benefit is the ability to control overabundant wild bird populations by non-lethal synthetic grit, similar in characteristics to what the bird uses in nature (VerCauteren et al. 2003). A chemical reproduction-inhibitor is encapsulated in synthetic grit that, upon ingestion, will break down in the gizzard and release the chemical for absorption. Additionally, grit physico-chemical properties and availability in various areas are major decisive factors for wild birds' (gallinaceous species and waterfowl) population density and geographic distribution (Owen & Cadbury 1975; Gionfriddo & Best 1996).

In fact, it was hypothesized that mineral-containing grit abundance or absence, impacted the welfare and distribution of some game bird species by supplying calcium to their deficient diet especially during early and reproductive stages (McCann 1939). Increasing growth rate (Scott & Heuser 1957; Spencer & Jenkins 1963), egg production (Balloun & Phillips 1956; Scott & Heuser 1957) and improving digestibility (Scott & Heuser 1957; Smith 1960) were also demonstrated in domesticated fowl. Conversely, Nestler (1946), Sibbald and Gowe (1977), Bennett and Classen (2003) reported no or very low benefit in using grit for confined domesticated birds and similarly no negative impact was detected in some grit-deprived gallinaceous (Nestler 1940) or companion birds (Taylor 1996).

1.4. Grit use by Different Avian Species:

As previously mentioned, grit use by different types of birds has been the subject of a multitude of studies and only a pertinent selection of these will be presented here. The following studies were conducted on waterfowl, gallinaceous, flightless, companion birds and poultry species.

Most research on waterfowl cast light on the relevance of grit use to lead toxicity (Gionfriddo & Best 1999). Waterfowl increased mortality is a serious issue caused by ingesting insecticide-treated seeds or pesticide granules as reported by Blus et al. (1984) and Wilson et al. (1998). While foraging for seeds and grit, waterfowl could involuntarily ingest lead shot that is similar in shape and characteristics to the grains, this will cause poisoning and eventually death (Sileo et al. 1973). Kimmel et al. (2007) reviewed the impacts of lead shot on wildlife which included "decreased survival, poor body condition, behavioral changes, and impaired reproduction". Bellrose (1959) concluded that when ducks retained lead shot for three or more weeks they died from its effects; hence retention time in the gizzard determined the degree of mortality within exposed birds. In contrast, grit availability and grit ingested amount could reduce lead shot long retention effect. It was inferred by Beer and Stanley (1965) that excess ingested grit passes rapidly through the birds, and could take lead shot with it.

Trost (1981) studied the factors affecting grit selection and retention in captive mallard in order to reduce the risk of lead exposure when mistakenly ingested. (Trost 1981) concluded that abundant grit availability in force-fed birds made the elimination time faster and the lack of grit caused longer retention in the lead-ingested duck, which eventually died from its effect. In the wild however, birds will not over-consume grit (if available) beyond their requirements to instinctively speed up the elimination of the lead ammunition. For that reason, dilution of shot-to-grit ratio in the wild or the use of a non-toxic alternative to lead shot will significantly mitigate the incidence of lead poisoning in birds (Kimmel et al. 2007). Another waterfowl mortality case (caused by ingestion of lost or discarded lead fishing materials) prompted the proposal of banning lead fishing sinkers of a size smaller than 25.4 mm (Franson et al. 2001). Accordingly, an analysis was conducted to evaluate the average particle size of ingested grit by two swan and one crane species to test the effectiveness of the ban. The findings showed a positive correlation between species body mass and grit mass, but not the size. No gizzard was free of grit and the largest particle was less than 20 mm with almost 90% of particles in the gizzards were smaller than 4.75mm. This proposed ban was considered efficacious until a subsequent study on a wider range of species showed that some birds had ingested lead weights larger than 25.4 mm and therefore the proposed ban would not prevent lead poisoning (Franson et al. 2003)

Nestler (1946) postulated that grit is dispensable for captive Northern Bobwhite quail and not essential for survival, growth, welfare, reproduction or gizzard development, as no differences or abnormalities were seen in the grit-deprived bird. However, a significant amount of grit was retained in birds' gizzards despite depriving grit-fed birds access to grit for 5 months. It was noted that hard seeds in the wild can be ingested and retained as a grinding agent, thus substituting for grit (Beer & Tidyman 1942).

Aganga et al. (2003) reviewed ostrich feeding and nutrition. Ostriches use grit stones in the gizzard to grind large particles, and those stones are never voided whole, only after being gradually abraded. The grit should be insoluble, and round with no sharp corners so as not to damage the gastro-intestinal tract. The presence of grit in the gizzard prevents digesta impaction which can lead to fungal and bacterial growth. Similarly, Waugh et al. (2006) evaluated the effects of grit on ostrich performance. Grit supplementation increased body weight gain and reduced feed intake compared to the no-grit group, which was explained by enhanced digestibility and improved overall feed efficiency.

Taylor (1996) evaluated the role of grit in the diet of canaries, which are common companion birds. The results of this study indicated that insoluble grit did not improve body weight or digestibility since no decrease in feed intake was noted for the grit-fed birds. The authors stated that canaries tend to ingest excess grit, that leads to impaction in the gastrointestinal tract and eventually the bird dies. However, when birds were denied the soluble grit (oyster shell) source, they consumed a higher amount of cuttlebone which is rich in calcium, and this behavior suggested a nutritional requirement.

Heuser (1946) in his book "Feeding Poultry" discussed the functions of grit in poultry diets and noted that the physical and chemical characteristics of the grit, will determine its usefulness as a grinding material or as a source of minerals. The author also emphasized the benefit of using sharp insoluble grit as a grinding agent when turkey poults or layer hens have access to coarse feed, structural material or whole grain, but it is not advisable to supply grit with an all-mash ration since it did not appear to increase feed efficiency.

Elliott and Hinners (1969) tested the effect of grit inclusion with different particle size of a corn based mash or pelleted diet. The authors concluded that grit did not influence growth or body weight gain but feed to gain ration was improved as feed particle size decreased regardless of grit addition. Grit retention time was affected by feed form, and was longer with pelleted diet.

Balloun and Phillips (1956) compared the efficacy of granite, quartzite and Sand River on the growth rate and feed utilization of broiler chicken. Birds showed no preference for color as the amount of grey granite and red quartzite consumed was almost the same. Sand River and the other two grit types equally improved body weight gain and FCR when an all-mash diet was fed compared to control group. However, grit supplementation improved egg production and FCR for adult caged hens when whole grain was mixed with the mash ration.

3. Materials and Methods

The field trial was conducted from the 29th of September until the 29th of October 2014 at Våler, Norway. A site visit was conducted on the 3rd of October by myself and Professor Birger Svihus to survey the conditions and provide additional instruction in relation to the experiment procedures.

3.1. Birds and Housing:

880 day-old unsexed broiler chicks with an average body weight of 43.65 grams were obtained from the Samvirkekylling hatchery. Birds were allocated randomly and equally to 11 floor pens (of 80 birds in each) covered with wood shaving and maintained on a commercial pelleted diet (with three dietary phases). The pens were distributed between four separate environmentally controlled rooms. Each pen contained either an automatic bell drinker or nipple drinkers with drip cups and a plastic bucket plate feeder. Feed and water were available ad-libitum throughout all the trial period. All birds were given a starter diet (0 to 10 days), grower (10 to 21 days) and finisher (21 to the end of the trial).

3.2. Grit Stones:

Dolomite Grits were initially acquired for the treatment group and 15 grams per bird were assigned to be fed between 4 and 11 days of age. However, we found that magnesium (Mg) concentration in dolomite is around 13% as reported by Roberts et al. (1990) (see **Table 1**), and when calculated, 15g dolomite contains (15g × 13%) = 1.95g (Mg) which surpasses even the layer's requirements of 0.55g "Mg" / 1000g Feed or 0.05% (NRC,1994). Hess and Britton (1997) investigated the effect of excess dietary magnesium on white leghorn hens and found it particularly detrimental to the bird's performance. Moreover, the estimated feed consumption in the first 11 days for broiler chicken is on average 332 g according to Aviagen (2007) and Cobb-Vantress (2013) broiler management guides, which is 3 times less than 1000g. As indicated by NRC (1994), (MgO) toxic dietary level for poultry is 5700 ppm or 0.57%. If dolomite was given to birds, the "Mg" concentration in feed consumed in 11 days would be $\frac{1.95}{332}$ =0.58%, in other word $\frac{0.58\%}{0.05\%}$ =11.6 times more than the requirement. The 0.58% exceeds the toxic level which would lead to retarded growth ,leg abnormalities and mortality (Lee & Britton 1980)

Consequently, granite grit containing a lower level of magnesium oxide, approximately 0.52 % (King et al. 1997) (see **Table 2**) or 0.31% "Mg" was procured from **Franzefoss,** a local supplier of gravel and crushed stone (stone aggregate), sieved to the proper diameter size and used instead .

Table 1: **Dolomite composition**

CaO	30.41%
MgO	21.86%
CO2	47.73%
Ca	21.73%
Mg	**13.18%**
C	13.03%
O	52.06%

Roberts et al. (1990)

Table 2 : Granite composition

SiO2	73.43%
Al2O3	13.35%
K2O	4.67%
Na2O	3.42%
CaO	1.04%
FeO	2.33%
Fe2O3	0.84%
MgO	0.52%
Mg	**0.31%**
TiO2	0.28%
P2O5	0.07%
MnO	0.05%

(King et al. 1997)

3.3. Treatment:

The trial consisted of seven control and four treatment (grit stones) groups. Three out of seven control pens were discounted at the end of the trial and thus excluded from data analysis. One pen had an early high mortality rate while the two other were removed randomly to equalize the number of pens.

During the first three days, all pens were managed under the same conditions. On day four, chicks from pens 1, 2, 9 and 15 were allocated to one single treatment where they were given grit stones on separate plates. Grit provision was initiated with 150 grams per pen, and continued on days seven, nine and eleven respectively with 350g per pen making it 1200g in total or 15g per bird, (**Table 3**). Three drinker rings (plates) were uniformly distributed in each cage with the amount of grit sprinkled and evenly divided among them. At day 18, the partially empty grit plates that remained were withdrawn from all pens, as the empty were removed previously.

Table 3 : **Grit provision design**

Age	**Pen 1** (g)	**Pen 2** (g)	**Pen 9** (g)	**Pen 15** (g)
4 days	150	150	150	150
7 days	350	350	350	350
9 days	350	350	350	350
11 days	350	350	350	350
Total (g)	**1200**	**1200**	**1200**	**1200**
g/bird	**15**	**15**	**15**	**15**

3.4. Data Collection

Production performance parameters like feed intake, body weight and mortality were determined throughout the field trial, while body weight gain and the feed conversion ratio were calculated at the end. Data such as internal organs' weight, grit weight and particle distribution were evaluated during laboratory analysis. Feed intake was recorded every time a feeder was refilled and the uneaten portion was collected and weighed at the end of each feeding phase. Body weights were measured at 10, 21 and 28 days of age. Mortalities were collected, weighed and recorded daily upon occurrence.

At 10 days of age, six birds were arbitrarily selected from each of the four treatment pens and weighed. Those birds were then killed by cervical dislocation, placed in labeled bags and frozen for later laboratory analysis. This procedure was repeated at 21 and 28 days consecutively, finishing with a total of 72 birds (**see Table 4**).

Table 4: **Randomly selected birds for lab. Analysis**

Age (days)	**Pen 1**	**Pen 2**	**Pen 9**	**Pen 15**	**Total** (birds)
10	6	6	6	6	**24**
21	6	6	6	6	**24**
28	6	6	6	6	**24**
Total (birds)	**18**	**18**	**18**	**18**	**72**

At 30 days of age, all remaining birds from the control and treatment groups were separately slaughtered at Nortura, Elverum. Following that, all gizzards from both groups were collected, divided into separate tagged bags (according to group) and frozen for a subsequent laboratory evaluation.

3.5. Lab Work:

The laboratory work was divided into three main parts. The first part was basically dry sieving the "as-fed" stones (to be offered to birds) to the proper size range and evaluating the particle distribution. The second part was dissecting the 72 birds with access to grit (18 birds × 4 pens), weighing of internal organs, emptying gizzard content, separating grit and weighing them individually. Particle distribution was carried out afterwards. The third part dealt with the rest of the gizzards of both groups which were divided previously in the slaughter house.

3.5.1. Initial Grit:

Earlier and recent studies by Spencer and Jenkins (1963), Koreleski and Swiatkiewicz (2004), Evans et al. (2005), Garipoglu et al. (2006), Adeniji (2009) and Bale-Therik et al. (2012) reported that the grit offered to broilers or layers had a size range of 1.5 to 4mm. At the supplier's quarry, stones and gravels were available in various size-ranges, however, the optimal range (1.5 - 4mm) that we aimed for was not available. Accordingly, the size acquired was between (2 - 6mm) and the grit were then sieved to the intended size range (1.6 -3.55mm).

A Retsch sieve shaker type (AS 200 Control) was used to sieve the stones. Amplitude chosen was 3.0 mm/g with one minute sieving time. Samples of 500g were screened through two test sieves with a screen aperture of 1.6 and 3.55mm. After collecting the screened sample, I ran another screening using seven test sieves to obtain a general view of how particles are distributed within the original sample. The seven sieves had a screen opening of 3.55, 2.8, 2.5, 2.0, 1.6, 0.8 and 0.5mm.

To calculate the percentage particle distribution, I used a Sartorius AX2202 digital scale balance (with 2200g capacity and 0.01g readability) to weigh the particles not passing through and collected on each test sieve (including the collecting pan). I then subtracted the sieve's full weight from the sieve's empty weight (measured previously). The differences in the values were divided by the total sample weight and multiplied by 100. Equation below:

$$\%\ \text{of particle of } n^{th} \text{ size} = \frac{\text{weight of } n^{th} \text{ test sieve full (g)} - \text{weight of } n^{th} \text{ test sieve empty (g)}}{\text{w. of sample (g)}} \times 100$$

3.5.2. Dissection:

12 birds per day were taken out of the freezer 15-16 hours prior to dissection and kept at room temperature. The next day, as carcasses were partially thawed it was convenient for dissecting and detaching organs easily. Carcass weight for each bird was taken and recorded before dissection. Next, the digestive tract (gut) from the proventriculus, gizzard, pancreas, small intestine (three sections) with its content, excluding the colon and ceca, was removed carefully to prevent any content escape (**Fig. 1**). Before weighing the gut, excessive fat surrounding the gizzard was scraped off using a blunt knife. The gizzard was then detached from the digestive tract, weighed full and empty after collecting its content. To separate the grit from the content, we tried a flotation method (decantation). This was a simple yet effective and rapid approach to split particles of different densities and made it easy to collect stones that sink from feed or wood particles that float. I emptied the gizzard content in a ceramic bowl and held it under a slow running faucet with a constant flow. The mixture was then disturbed and low density particles floated and flowed out of the bowl (**Fig. 2**). The procedure took about four minutes per gizzard. Stones were then placed in a small translucent container coded by cage, sample number and age on the lid (1-1-21) and left overnight at room temperature to dry and be weighed individually the next day. The above procedure was repeated for all of the 72 samples after which the following steps were followed. The six samples (grit extracted) from each of the four pens at 10, 21 and 28 days of age (refer to data collection section) were pooled together for a particle size evaluation. In total, we had 3 samples per pen at three ages or 12 samples (**Table 5**).

Fig. 1 : **A** : Gut from proventriculus to colon;
B : Ceca and colon separated, fat surrounding gizzard removed.

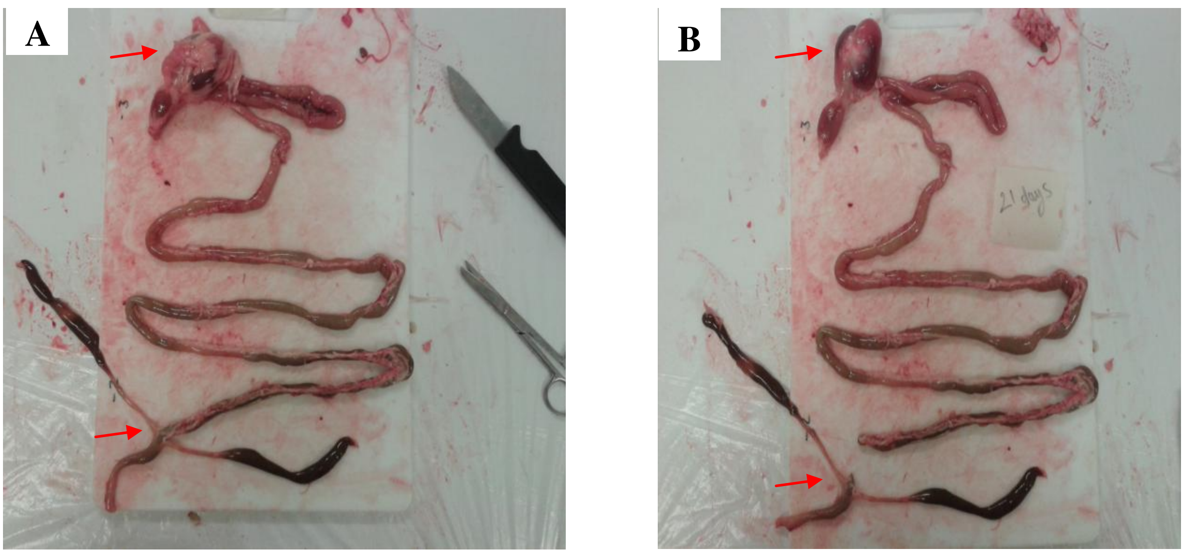


Fig. 2-- Decanting Stones From Gizzard Contents.

A : Gizzard content + grit
B : Slow running faucet disturbing the mixture
C : Low density particle floating
D : Final rinsing

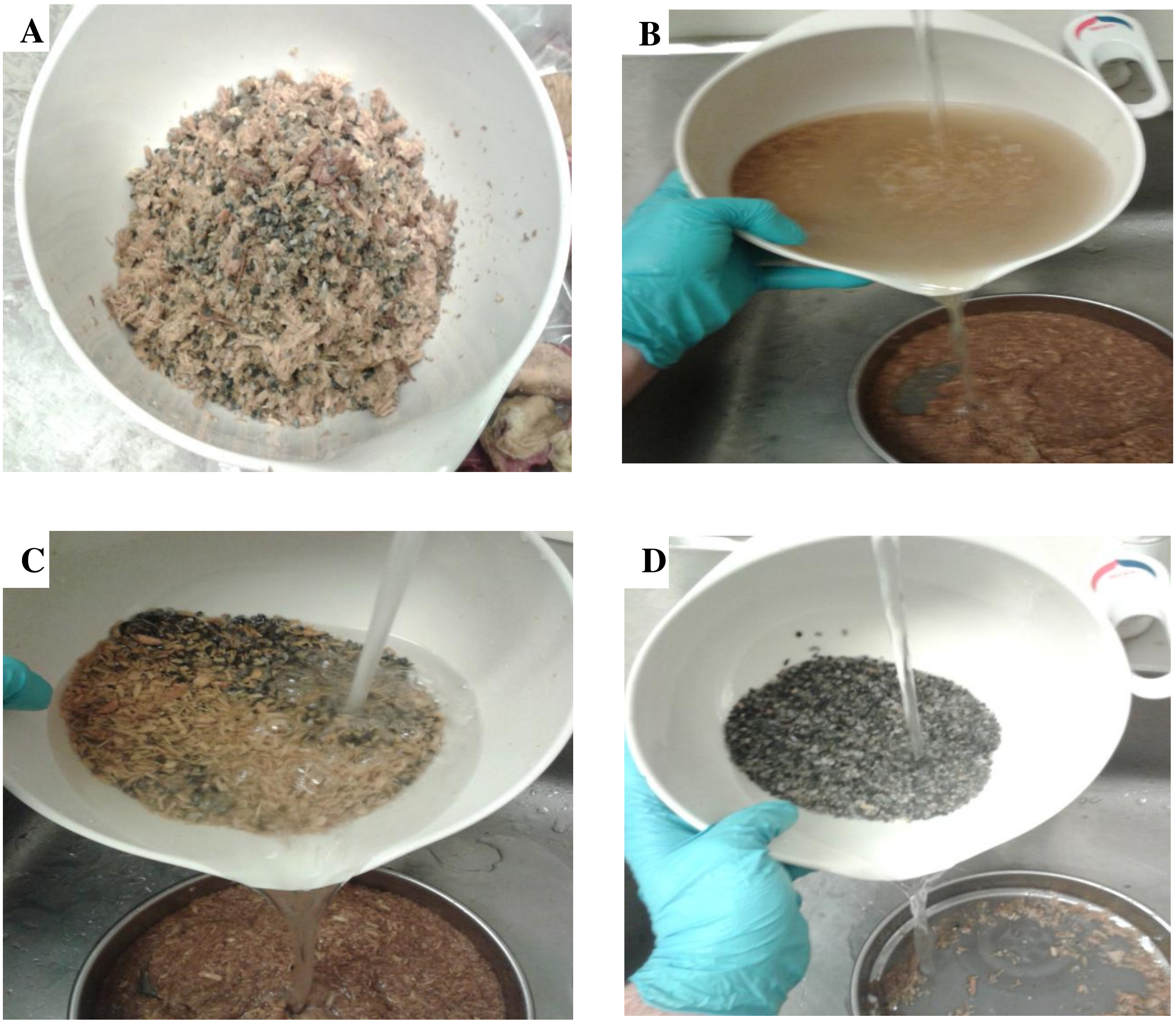
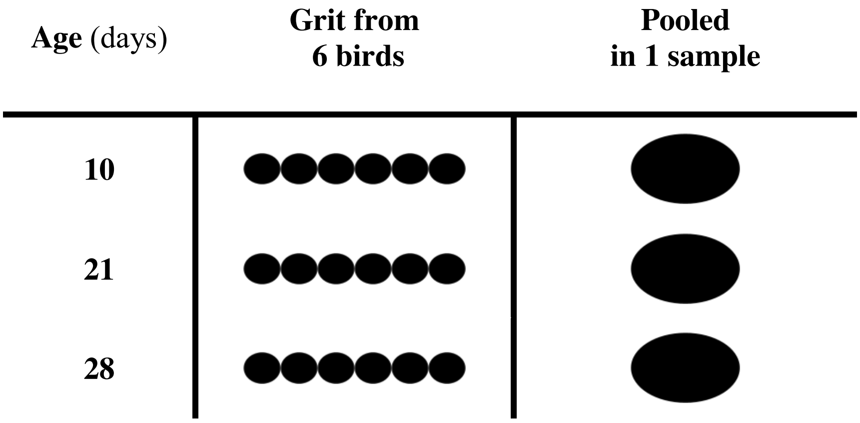


Table 5 : **Pen 1**

Pooling grit for Particle distribution analysis



Particle distribution was carried out in the same way as in Part 1. The series of the seven selected test sieves had similar screen aperture with opening decreasing from top to bottom. The reason for selecting seven sieves was to gain a more comprehensive view of the modification in particle size distribution when compared with the original stones.

3.5.3. Slaughter House Gizzards:

One of the objectives in this field trial was to investigate the quantitative variation of grit stones between birds. For practicality, I randomly selected 72 from the 208 gizzards from the GG (slaughter house), weighed them full and empty, separated the grit and weighed their amount individually. In addition, and for better interpretation, statistical comparison for the rest of the gizzards, required individual weighing, full and empty. Lastly, the grit stones gathered were pooled together and analyzed for particle size distribution.

3.6. Statistical Analysis:

Birds' performance and the 416 gizzards weight data, were subjected to a one-way ANOVA using SAS software.

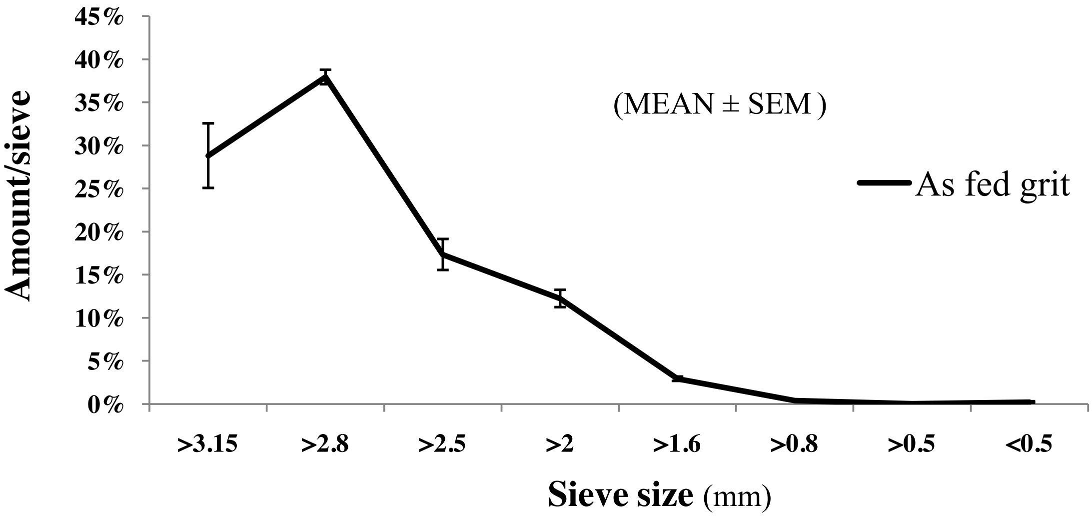
A regression analysis was as performed using Excel (2007) to test the relationship between grit weight and gizzard empty weight at different ages.

4. Results:

4.1. Initial (as-fed) Grit Particle Size Distribution:

Initial grit particle size distribution is illustrated in **Fig. 3**. As it was described in part 1 (lab work), as-fed grit was sieved to yield a particle size range of 3.55 to 1.6mm. The graph shows clearly that almost 99.25% of the grit fell within this range and the highest proportion was for coarser grit (3.15 and 2.8 mm) that made up 29% and 38% respectively. In contrast, medium and smaller particles combined, only accounted for one-third of all the sample.

Fig. 3-- Particle Size Distribution: Initial (as-fed) Grit.



4.2. Broiler Performance Parameters:

Broiler performance parameters (body weight gain 'Bwg' and feed intake per bird) for the grit group (GG) and no-grit-group (NGG) during the starter (0-10 days), grower (10-21 days), and finisher (21-28 days) growth periods are presented in **Table 7**. Adjusted feed conversion ratio 'FCR', corrected for mortality and calculated by dividing the body weight gain by its respective feed intake, is also shown. In the bottom row, p- value represents the probability of obtaining the observed outcome by chance and allows for a better statistical interpretation and comparison between treatments. Low *p*- value ($P < 0.05$) means that the observed effect is unlikely to have arisen by chance and therefore a significant difference between treatments exists.

No significant difference ($P > 0.05$) between treatments was observed in Bwg or feed intake during the starter phase. Nevertheless, there was a tendency for an improved FCR for grit-fed birds ($P = 0.068$) compared to the NGG. The tendency for better FCR was influenced by a slight but not significant lower feed consumption and higher Bwg in the GG. SD for Bwg, feed intake and FCR are negligible (Coefficient of Variation, CV% $= \frac{SD}{Mean} \times 100$, were < 5% for each parameter, suggesting a good weight uniformity of chicks/pen/treatment at placement. Even though Bwg SD (±4.5) was double for grit-fed birds compared to the NGG Bwg SD (±2.4), this difference is nonetheless not considered significant because the period is very short and weight gain variation is considered trivial.

No significant difference ($P > 0.05$) between treatments was observed in Bwg, feed intake or FCR during the grower phase. In addition, variation was negligible for all parameters (CV ≤ 5%) denoting a satisfactory uniformity of chicks in both treatments. However, a higher (4×) Bwg variation SD (±31.2) (**Table 7**) in the NGG in comparison with SD (±7.8) in the GG was noted due to a lower Bwg (675g) in pens18 (**Table 6**) compared to the three other, even though feed intake was not affected. This higher variation affected FCR since the latter is derived from Bwg and feed intake.

There was no observed significant difference ($P > 0.05$) between Bwg, feed intake or FCR in both treatments during the finisher phase. Birds showed consistent performance in both treatments as

Bwg, feed intake and FCR did not vary considerably (CV ≤ 5%) (**Table 7**). However, Bwg for grit-fed birds SD (±32.8) varied noticeably in comparison with the previous phase (SD ±7.8).
This was due to a steady slow growth in pen1 (**Table 6**) associated with a constant lower feed intake which accumulated over 3 growth periods and resulted in a higher deviation compared with the three other pens where difference was more or less consistent over time.
It should be mentioned that pen1 (**Table 6**) had the highest mortality rate. Furthermore, a high variation (SD ±34.9) in **Table 7** was also seen in Bwg in the NGG due to pen18 in **Table 6** where birds exhibited an accelerated compensatory growth and achieved the highest body weight gain (740.39gr).

Table 8 illustrates broiler cumulative performance data for the overall 0-to-28 days trial period. No significant difference ($P > 0.05$) was noticeable in any of the performance parameters (Bwg and feed intake) between treatments. Neither Bwg nor feed intake were significantly affected by grit provision to birds. In comparison with the NGG, mortality rate was numerically 25% lower (2.52 vs. 3.13%) for the grit-fed birds, statistically however this difference was not significant ($P > 0.05$).

While access to grit did not seem to harm nor improve overall birds' performance, higher variations in cumulative Bwg and feed intake were observed (**Table 8**). Yet, in **Table** 7, NGG had a higher standard deviation in the middle and last period as exemplified in pen18. But the deviations produced by this pen were moving in opposite directions and hence evened each other out, resulting in a very low variation at the end of the rearing period. On the other hand, GG showed relatively lower variation in the last two periods and this was justified previously by the consistent difference between the pens. Unlike pen18, pen1 in **Table 6** was characterized by a constant lower Bwg ascribed to a lower feed consumption causing an accumulation over three growth periods and eventually higher variation. In contrast, pen2 and pen15 (**Table 6**) experienced an opposite growth pattern where parameters values moved positively. Thus, the overall variations did not cancel each other out and a wider distribution in Bwg and feed intake was seen for GG.

Table 6-- Pens Performance Parameters During Three Consecutive and the Overall Period.

		Starter 0-10 days			Grower 10-21 days			Finisher 21-28 days			Overall experimental period 0-28 days			
Pen	Treat.	**Bwg** [1]	**FI** [2]	**FCR** [3]	**Bwg**	**FI**	**FCR**	**Bwg**	**FI**	**FCR**	**Bwg**	**FI**	**FCR**	**Mort %**
1	**Grit**	271.05	274.69	1.01	718.32	1010.21	1.41	655.79	1017.94	1.55	1645.17	2302.84	1.40	**7.5**
2		275.10	285.63	1.04	736.00	1033.04	1.40	732.00	1081.03	1.48	1743.10	2399.69	1.38	**0**
9		274.19	293.13	1.07	732.00	1022.95	1.40	692.00	1041.87	1.51	1698.19	2357.94	1.39	**1.25**
15		281.69	293.75	1.04	725.00	1038.70	1.43	714.00	1066.57	1.49	1720.69	2399.02	1.39	**1.25**
7	**No grit**	270.76	284.50	1.05	732.44	1023.35	1.40	725.44	1062.08	1.46	1728.65	2369.93	1.37	**5.0**
8		273.21	293.00	1.07	738.12	1028.46	1.39	676.00	1046.23	1.55	1687.33	2367.70	1.40	**3.75**
18		275.90	310.94	1.13	675.00	1047.13	1.55	740.39	1036.88	1.40	1691.29	2394.94	1.42	**2.5**
26		275.50	299.81	1.09	740.26	1033.81	1.40	671.00	1060.13	1.58	1686.76	2393.75	1.42	**1.25**

[1]Body weight gain (gr); [2] Feed intake per bird ; [3] Adjusted feed conversion ratio (corrected for mortality)

Table 7-- Treatments Average Performance Parameters of Broilers During 3 Consecutive Periods (MEAN ± SD[1]).

	0-10 days			10-21 days			21-28 days		
	Bwg	**FI/bird**	**FCR**	**Bwg**	**FI/bird**	**FCR**	**Bwg**	**FI/bird**	**FCR**
Grit	275.5 ± 4.5	286.8 ± 8.9	1.04 ± 0.02	727.8 ± 7.8	1026.2 ± 12.5	1.41 ± 0.02	698.4 ± 32.8	1051.9 ± 27.8	1.51 ± 0.03
No grit	273.8 ± 2.4	297.1 ± 11.2	1.08 ± 0.03	721.5 ± 31.2	1033.2 ± 10.2	1.43 ± 0.08	703.2 ± 34.9	1051.3 ± 11.9	1.50 ± 0.08
P-value [2]	0.5891	0.1957	0.0686	0.6988	0.4413	0.5593	0.8565	0.9621	0.8399

[1]Standard deviation
[2]*P-value* > 0.05 indicates no significant difference

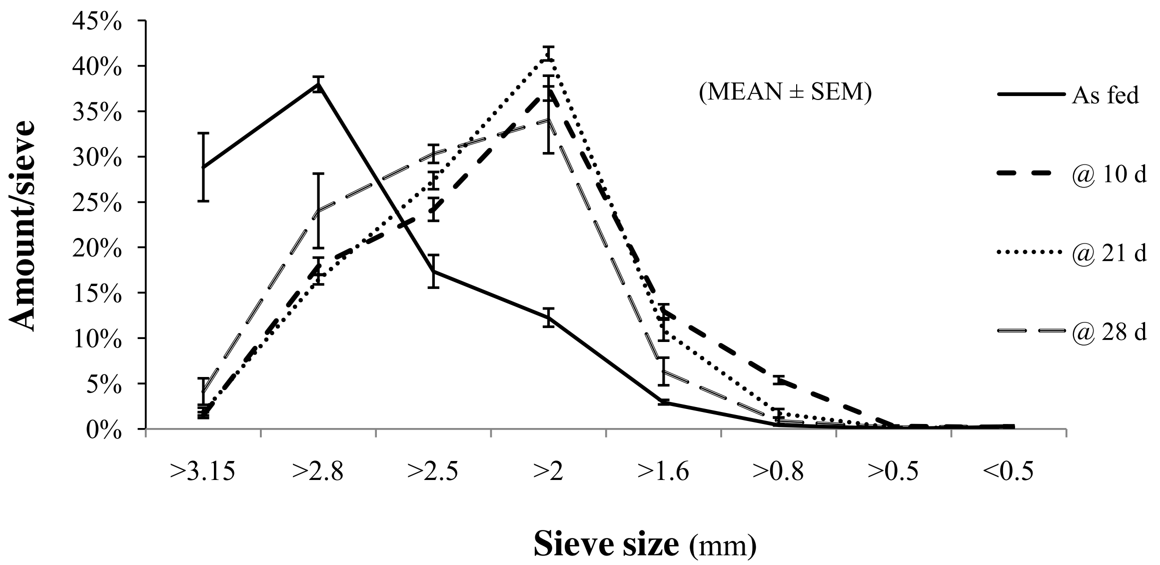
Table 8-- Treatments Cumulative Performance Parameters of Broilers Throughout The Experimental Period.

	0-28 days			
	Bwg	**FI/bird**	**FCR**	**Mortality**
Grit	1701.8 ± 41.9	2364.9 ± 45.7	1.39 ± 0.01	2.50%
No grit	1698.5 ± 20.2	2381.6 ± 14.8	1.40 ± 0.02	3.13%
P-value	0.8940	0.5118	0.3473	0.7502

4.3. Initial and Grit-Fed Particle Size Distribution:

A graphical comparison of combined particle size distribution results is presented in **Fig. 4**. In contrast to initial grit, grit collected from gizzards at different ages graphically showed a clear shift in peaks towards medium to small size particles, with a sharp reduction in larger particles. The percentage of medium and small grit (2.5, 2 and 1.6mm) increased from 30 % to more than 70% as opposed to larger particles which dropped to about 30%. No significant difference in amount of particles smaller than 0.8mm was noticed.

Fig. 4-- Particle Size Distribution of Grit Collected from the 24 Birds Killed per Age.



4.4. Dissection:

Table 9 shows that gut and gizzard relative weight decreased with increased body weight. Full and empty gizzard relative weight decreased by almost 50 % from 10 to 28 days. Of the quantity offered, grit retained in the gizzard represented 25, 32 and 22 % at 10, 21 and 28 days respectively. Variation in grit retained increased considerably with age. At 10 days, grit filled more than one-third of the gizzard content and decreased to one-fifth at 28 days.

Table 9-- Dissection Results for the 24 Grit-Fed Birds Killed per Age.

	Weight	Bird (g)	Gut [2]	Gizz.[3] Full [2]	Gizz. Empty [2]	Gizz. Content (g)	Gizz. Stone (g)	Stone % of Content
10 days	Avr.	336.40	14.7 %	4.8 %	2.7 %	7.17	2.64	36.78
	±SD.[1]	34.68	1.52 %	3.05 %	0.37 %	1.64	1.01	10.78
	CV[4]	10.3%					38.4%	
21 days	Avr.	1038.45	9.83 %	3 %	1.7 %	13.19	4.83	36.59
	±SD.	168.48	1.51 %	0.58 %	0.27 %	3.66	2.17	13.68
	CV	16.2%					45.1%	
28 days	Avr.	1699.50	7.86 %	2.3 %	1.32 %	15.69	3.31	21.07
	±SD.	181.30	0.91 %	0.35 %	0.13 %	3.95	2.55	16.78
	CV	10.6%					77.1%	

[1]Standard deviation
[2] % of body weight
[3]Gizzard
[4]Coefficient of variation

4.5. Effect of Grit Amount on Empty Gizzard's Weight.

At 10 and 21 days (**Fig. 5 & 6**), there seemed to be a relationship between the amount of grit stones and size of the empty gizzards, but not at 28 days (**Fig. 7**).

Fig. 5- Gizzard vs stones weight at 10 days

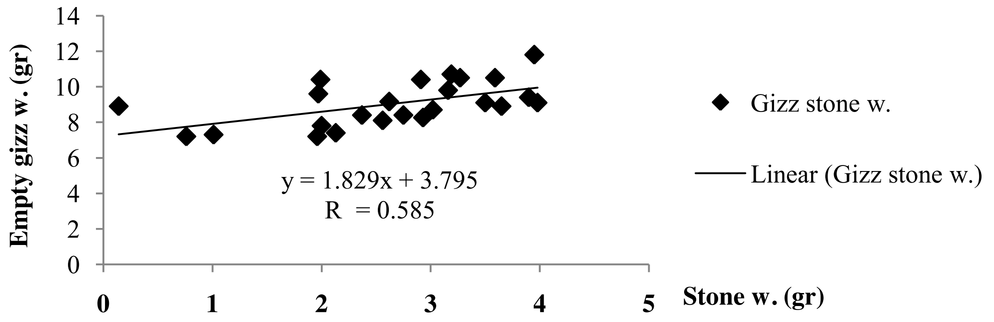


Fig. 6- Gizzard vs stones weight at 21 days

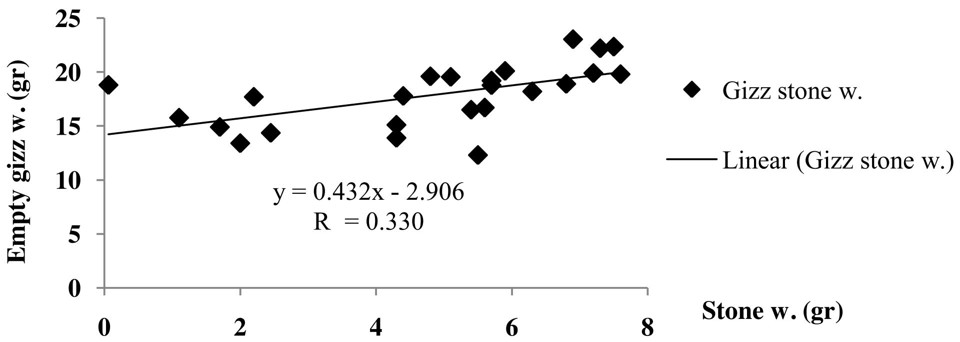
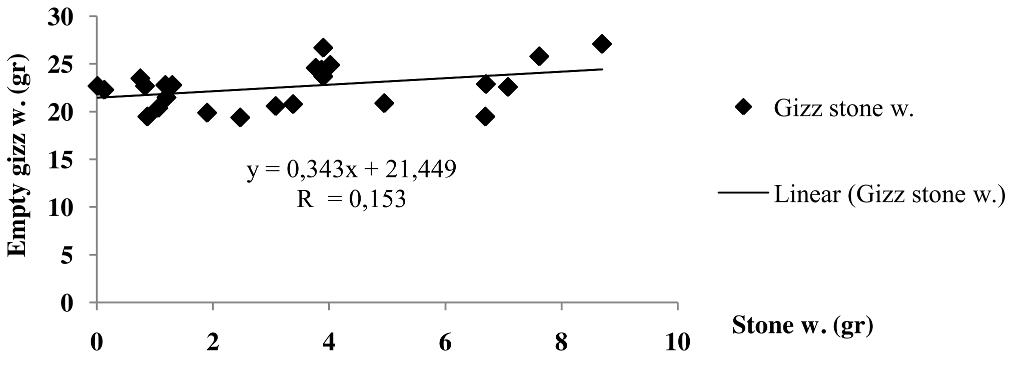


Fig. 7- Gizzard vs stones weight at 28 days



4.6. Weight Distribution of Grit Collected from the 24 grit-fed birds killed per age.

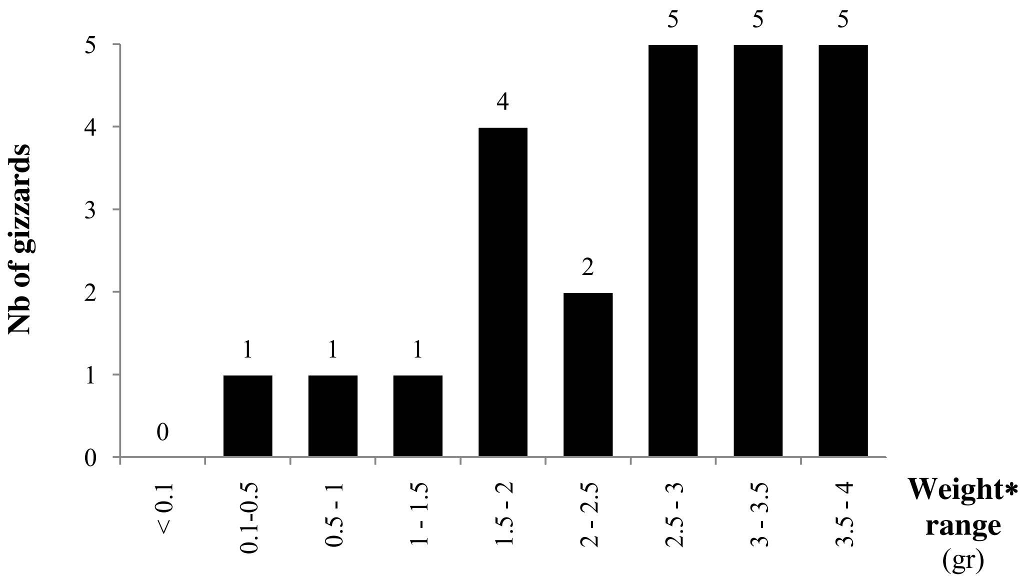
At 10 days of age (**Fig. 8-A**), all birds had grit in their gizzards and the majority retained more than 14 % (in weight) of the grit offered during this period. The variation (CV=38%) is fair compared to the following growth periods, since 62% of the birds retained close grit weight-ranges as seen by the summarized data to the right side.

Similarly to the first growing period, no birds at 21 days had no grit in the gizzard (**Fig. 8-B**). The variation (CV=45%) increased since the range of grit weight became wider. Birds showed dissimilar retention behavior but in general, 75% had more than 4 gr of stones.

The 28 days chart (**Fig. 8-C**), is somehow the mirror image of that at 21 days as the values are summarized at the left side (less retained grit). The greater dispersion in the values indicates substantial variability (CV=77%) in grit retention and elimination in broilers as age increases. 70% of the birds had (=<4 gr) of stones.

Fig. 8-- Weight Distribution of Grit from the Dissected Birds at Different Ages.

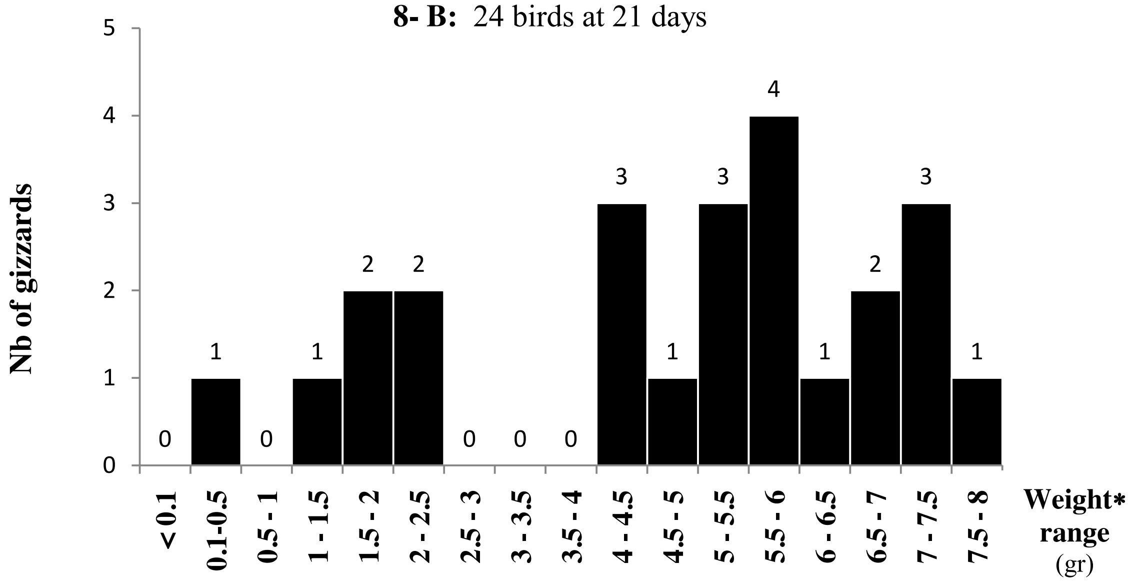
8- A: 24 birds at 10 days



* Weight range of (X - Y) means X <weight< Y

Avrg.= 2.6 gr ; SD = 1.01 ; SEM = 0.21

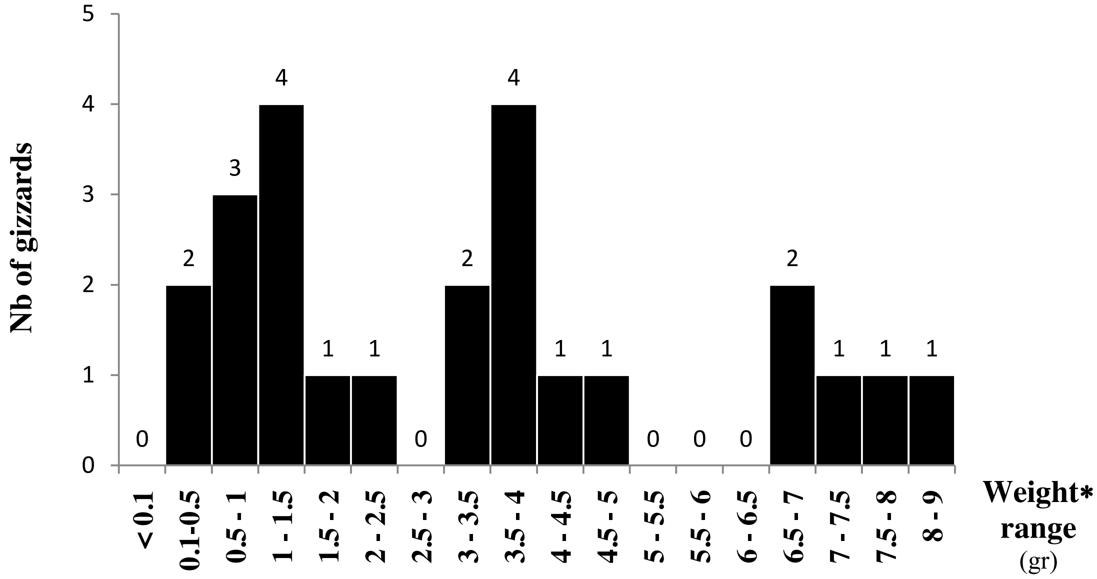
8- B: 24 birds at 21 days



∗ Weight range of (X - Y) means X <weight< Y

Avrg.= 4.8 gr ; SD = 2.17 ; SEM = 0.44

8- C: 24 birds at 28 days



∗ Weight range of (X - Y) means X <weight< Y

Avrg.= 3.31 gr ; SD = 2.55 ; SEM = 0.52

4.7. Weight Distribution of 416 Gizzards at 30 days:

Box plot is a graphical way to see how data is distributed (McGill et al., 1978). The small square inside the box represents the mean. The horizontal line inside the box (not necessarily in the center) indicates the median value. The median separates the higher half of a data set from the lower half, into two equal parts (50% each). The spread or concentration of values (above or below the median) in a data set will depict the symmetry or skewness of the box plot parts. Each part is divided into quartiles or (25% of the data). The inter-quartile range (IQR) is the distance between the first and the third quartile (edges of the box), or the height of the colored box. The colored area of the box shows 50% of the total data. The whiskers or lines extending out from the box show the minimum (extends from first quartile downwards) and maximum weights (extends from third quartile upwards). Outliers are extreme values which lie above or below more than 1.5 times the length of the box; these are plotted separately as clear points.

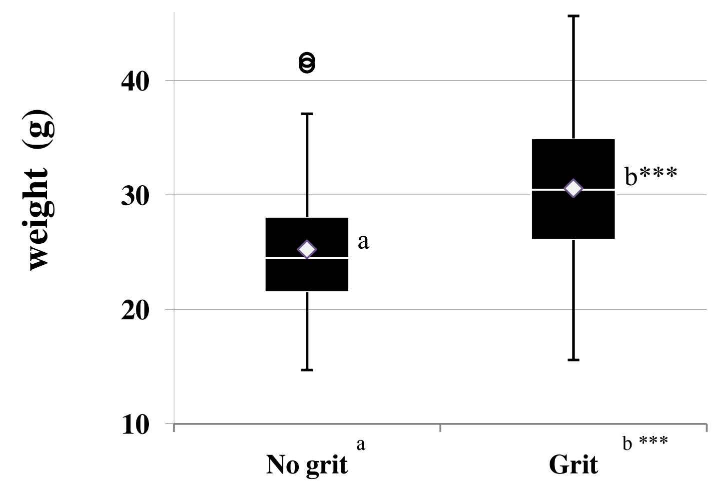
Fig. 9- A: The two box plots show symmetry in the data as the mean is located at the center of the boxes and divides it equally. Mean full-gizzard-weight in the grit-group (GG) was significantly higher ($P < 0.001$) (almost 22%) than that of the no-grit-group (NGG). The spread of values in the GG data set has a wider range (distance from bottom to top whiskers) which indicates more dispersion in the data. The IQR in GG has a wider distance (as shown by the lengths of the box) indicating a relatively higher variation in gizzards' weights. Over 75% of the full gizzards in the GG were heavier than half of those from the NGG.

Fig. 9- B: The IQR are more or less similar indicating similar variation in empty gizzards' weights. Mean empty gizzard weight in the GG was significantly higher ($P < 0.001$) (almost 8%) and almost 75% of the same group were heavier than half of the gizzards in the NGG.

It was observed during dissection that out of 208 gizzards in the GG, 17 gizzards or 8% of the total contained no grit. Seven of the 17 were at least 1 standard deviation (SD) smaller than the total average empty weight, while the other 10 were within 0.5 SD from the average.
Seven gizzards or 3% of the 208, contained very few grit (less than 0.5gr or 25 stones), and had a lower empty weight compared to the total average empty weight. Two out of the seven gizzards however, were enlarged and heavier than the total average empty weight.

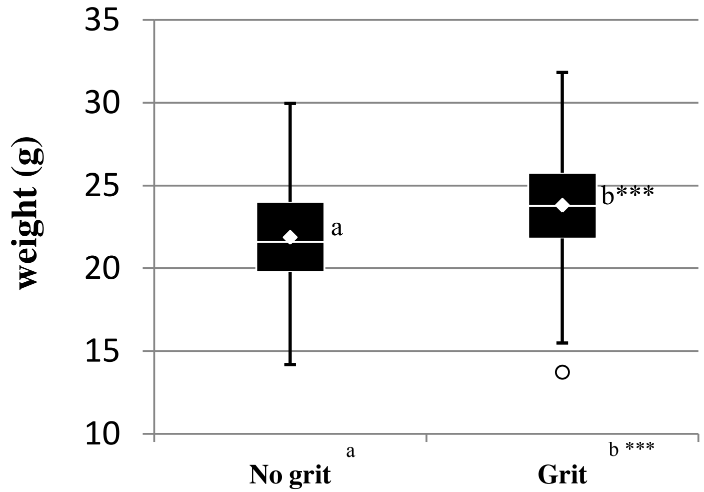
Fig. 9-- Box Plots Showing Weight Distribution of 416 Gizzards at 30 Days.

9-A: Full gizzard[1]



NGG Mean ± SD: 25.08 ± 4.74; GG Mean ± SD: 30.59 ± 6.39
(2 outliers in NGG)

9-B: Empty gizzard[1]



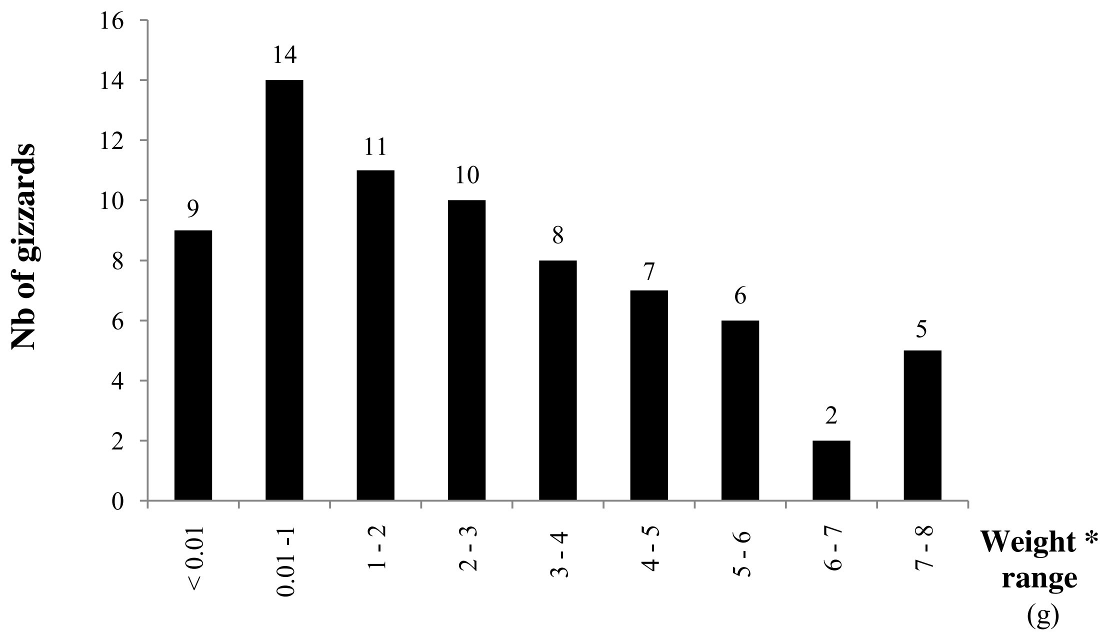
NGG Mean± SD: 21.87 ± 3.08; GG Mean ± SD: 23.8 ± 3.32
(1 outlier in GG)

[1] 208 gizzard in each treatment; 416 in total
[a b] Means without a common superscript differ significantly
Level of significance: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

4.8. Weight Distribution of Grit from the 72 Randomly Selected Gizzards at 30 days:

At slaughter age, only a small number of gizzards contained very few stones but nearly 70 % of the 72 randomly selected gizzards had more than 1 gr (6% of the original amount) which is equivalent to about 50 stones (estimated after measuring the particle distribution at 30 days). Also we can see that the number of birds with larger amounts of stones declined gradually toward the end of the trial (**Fig. 10**)

Fig. 10 -- Weight distribution of grit from randomly selected gizzards.



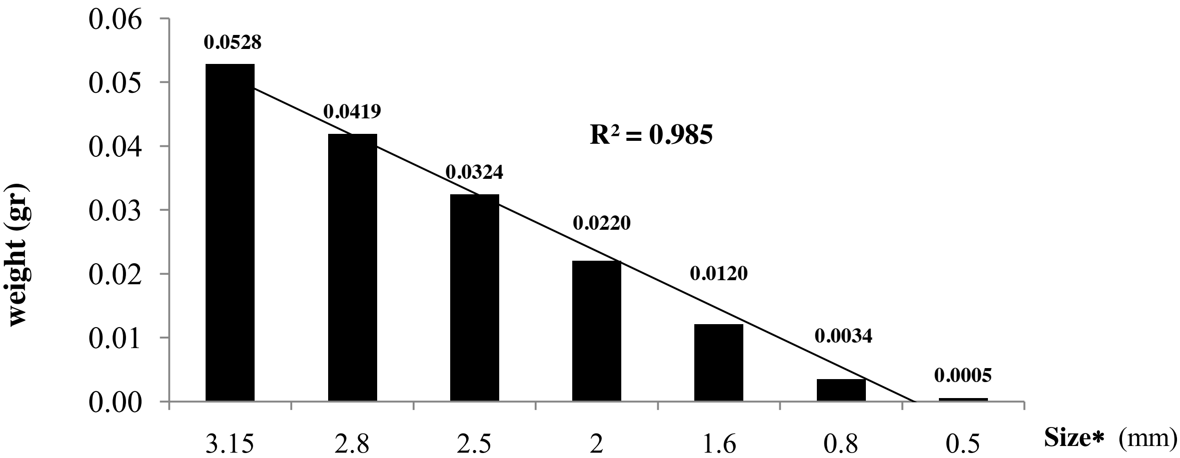
∗ Weight range of (X - Y) means X <weight< Y

Avrg.= 2.66 gr ; SD = 2.31 ; SEM = 0.27

4.9. Size and Weight of the Grit:

There was a linear relationship between grit size and weight (**Fig. 11**), suggesting limited erosion on particles and accordingly shape was approximately the same, independent of grit size. More blunt edges compared to the irregularities and sharp corners of the initial grit (see **Appendix**).

Fig. 11- Relationship between grit weight and size.



* Weight of a stone within a size range is X > weight > Y
Ex: weight = 0.0419
Stone size is between 3.15 > size > 2.8mm

4.10. Mean Grit Count at Different Ages:

In the current study, the maximum number of stones found was 243, 366 and 368 in a 10, 21 and 28 day-old bird respectively. It is evident that when all the assigned grit was offered (15g by 11 days of age), birds had the largest number retained (at 21 days) and when grit access stopped, reduction in the gizzard grit count was noted. With age, a higher and wider variation in the amount of grit retained was recorded (**Table 10**).

Table 10- Average Number of Grit from the 24 Birds Killed per age.

Age	10 days	21 days	28 days
Mean ± SEM	161 ± 12.65	231 ± 21.38	140 ± 22.01
SD	62	104	108

5. Discussion:

5.1. Birds Performance:

In general, grit-fed birds' performance was not impaired nor were there any observed beneficial effects during growth periods. Similar results have been found before by Kennard and Chamberlin (1959), Hollister (1991) and Garipoglu et al. (2006) who reported no effect for grit supplementation on any of the birds' performance parameters. Although mash or whole grain ration were used in most of the studies on the effects of grit supplementation, there were conflicting findings as the results were inconclusive. These included an improvement in performance (Fritz 1937; Balloun & Phillips 1956; Scott & Heuser 1957; Smith & Macintyre 1959), an adverse effect (Arscott et al. 1955; Majewska et al. 2009), and no observed benefit (Piatt & Stephenson 1935; Fuller 1958; Salverso & Little 1966; Sibbald & Gowe 1977; Bennett & Classen 2003).

At four days of age, grit was offered separately on three red plates in each cage. Both the red color of the plate and the grayish black grit attracted the birds and triggered their curiosity to start pecking. Shortly after, plates became crowded as almost all birds showed a strong appetite; consequently all grit was eaten. By 10 days of age, grit-fed birds received 70% of the total intended grit amount per pen, although no difference ($P > 0.05$) was observed in feed intake per bird in both treatments. As a result of this similar feed intake, body weight gain for grit-fed birds did not decrease, rather it was similar to the no-grit group.

In contrast to Hinners and Elliott (1972) belief, grit in this study did not compete with the feed for gizzard space and thus did not impede normal nutrient absorption rate as feed intake was not reduced even at an early age. However, the fact that grit stones will take up space in the gizzard, logically implies a decrease in feed holding capacity in the gizzard. Therefore, in order for the grit not to reduce feed intake, there must be a compensatory mechanism or a possible combination of factors that may partially or to a lesser extent prevent this reduction.

By intuition, the first factor that most likely countered reduced feed consumption was the increase in gizzard's volume caused by the effect of stones as structural components on muscle stimulation (Spencer & Jenkins 1963; Svihus 2011a; Sacranie et al. 2012). In addition, presence of hard stones promoted grinding activity in the gizzard as reported by Hetland et al. (2003) thus increasing smaller particles proportion which was reported to have a fast passage rate (Nir et al. 1994). A second, less likely factor is feed form where, pellet granule disintegrates rapidly inside the upper gastrointestinal tract (GIT) when moistened and its fine particles pass quickly down the tract (Engberg et al. 2002; Svihus 2006; Amerah et al. 2008; Buchanan et al. 2010). Although plausible and observed during laboratory analysis, grit passing rapidly through the gizzard is less likely a direct factor since dissection results showed a significant amount of grit retained in the gizzards even 12 days after withdrawal (see **Appendix**). Furthermore, the amount of ingested grit (overconsumption) can also play a role and may interfere with the gizzard grinding mechanism or space for food, although no mortality was recorded from an excess grit intake.
It is worth mentioning that in two out of four pens, a small amount of grit remained unconsumed even at 18 days (and eventually was taken out). However, it is possible that the quantity offered and/or eaten by the birds is more than needed for optimal gizzard function, and hence could influence further feed utilization. Finally, grit particle size will also be discussed since it can directly affect gizzard volume and available space for food.

As discussed in the literature review section, the enlargement and subsequent increase in gizzard weight is attributed to more extensive grinding activity needed to reduce the large particle as it is retained longer in the gizzard. This observation was anticipated since the presence of hard stones in the gizzard required further grinding activity for particle reduction, and correspondingly increased gizzard compartment volume. Increased gizzard's volume suggests more room and less obstruction inside the gizzard. More space means that the gizzard can accommodate additional feed quantity and less obstruction implies a normal passage rate for feed particles. Shires et al. (1987) detected a positive correlation between feed passage rate and nutrient intake. As the birds in both groups consumed equal amounts of feed and had similar body weight gain, we can conclude that grit-fed birds adapted to the presence of grit in their stomach by increasing the gizzard volume, and as a consequence, feed passage rate and nutrient uptake behaved similarly in both treatments.

Moore (1999) suggested that in geese, increased passage rate of food particles (grass) would be achieved either by additional particle size reduction or by elimination of intact large particles. Elimination of large food particles in this study is not likely since food intake was similar and FCR would have been larger if grit-fed birds were using more feed for the same weight gain. Therefore, additional particle breakdown aided by the stones is a more logical explanation.

Moore (1998a) explained the breakdown process of the grass by the stones' large surface area and thus larger damaging sharp edges contacting the food. Also, Gionfriddo and Best (1999) indicated that providing insoluble hard grit to the bird will offer dynamic and grinding surfaces to food in the gizzard.

According to Hetland et al. (2003), grit supplementation for broilers improved grinding efficiency as it was expressed by the significant decrease (P = 0.023) in duodenal mean particle size. Contrarily, oat hull and whole grain addition to diet in the same study, did not have any effect on particle reduction. Subsequently, an increase in particle passage rate will be expected since Nir et al. (1994) reported a negative correlation between passage rate and particles size. The larger the particle, the slower its passage and the smaller the size, the faster it passes.

Efficacy of grinding is dependent on the grit hardness as discussed by Buckner and Martin (1922) and Smith and Macintyre (1959). The authors stated that hardness, durability and insolubility are the main characteristics of grit if used as an effective grinding agent. Consequently, insoluble, acid resistant grit like granite, quartz and silica would be superior to soluble materials like oyster shell or limestone grit. The authors also hypothesized that insoluble grit can function as grinding material only until they lose their identity as distinctive particles.

The materials used in this study was granite grit which has a hardness of "7" as given by Mohs' Scale compared to calcite "3" and Diamond "10" (Cordua 1998). The particles collected from gizzards during dissection had more or less the same shape with smoother blunt edges which proves their resistance to acid and abrasion, although more particles (>2.8 and 3.15mm) were ground and disappeared due to the intense gizzard grinding activity.

Since it was not the focus of this thesis, neither crop size nor its content were examined during dissection. However, several studies confirmed a fast disintegration of the pellet granule in the upper digestive tract when compared with mash or whole grain. Engberg et al. (2002) for instance, attributed the lower gizzard weight to its low dry matter content when pellet feed was offered since the mechanical stimulation of the feed particles was not adequate. The authors concluded that as pellet dissolves fast, it passes through the upper digestive tract to the small intestine quickly whereas mash or coarse ground mash occupy the gizzard for a longer time because of the extensive need for grinding to the critical size before they can pass through. It is noteworthy to consider the effect of the pellet press on particle size reduction. Svihus et al. (2004b) concluded that the pelleting process evened out differences in particle distribution due to the additional grinding action of the rollers in the pellet press. As a result, the pellet formed will contain more finely ground particles than mash or coarse mash. Therefore, when pellet dissolves in the upper tract, fine particles will pass through at a faster rate than coarser particles.

McIntosh et al. (1962) examined the interaction of whole, ground and pelleted grain with grit stones. In their work, the authors detected an increased metabolizable energy value when grit was supplied to whole, ground or pelleted grains although with whole grain, the effect on ME was greater. It was then hypothesized that the extended grinding action of the grit when whole grain was fed caused greater enhancement in nutrient utilization compared to the other forms (longer retention time and contact with enzymes). However, since an improvement in ME with the ground and pelleted grain was seen (even though lower than whole grain) a suggestion was made that a compensatory factor for the limited grinding time associated with those two forms is present. McIntosh et al. (1962) speculated that grit accumulation may stimulate the production of more digestive juices, slows down the passage rate of the feed with a simultaneous mixing with the acid and enzymes allowing for a complete digestion.

In combination with the enlarged gizzard volume (previously discussed), it can be postulated that the short retention time of fine particles in pelleted feed in the upper part of the GIT and its rapid passage did not decrease feed intake, which is in accordance with Shires et al. (1987) who found that a retention of digesta in the crop and gizzard may reduce the rate of feed intake.

It was observed during dissection, that some stones (at different ages) were obviously passing through the gizzard to the small intestine unground and thus being eliminated at fast a rate (see **Appendix**). This rapid elimination process would have been a determinant factor of feed consumption if the grit quantity at the end of the trial was less than what was actually found among birds. Even after 12 days of no grit access, a significant number of gizzards had a lot of grit which justifies the non-contribution of this factor in feed consumption rate. On the other hand, if this was to be tested, the GIT of each bird should be examined individually at different time intervals to be able to confirm the existence of any relationship. This is tedious and considered impossible.

Since birds rely on their gizzards to macerate and triturate food for further digestion, an efficient grinding mechanism is therefore required. As reported by Svihus (2011a), the thick and thin gizzard muscles are asymmetrically arranged, and upon contraction a rotary as well as crushing movement result. Moore (1998b) described the contraction movement as translational which is manifested when gizzard walls contract and simultaneously move in opposite directions. This combined pressure and translational movement create shear and spread damage across gizzard contents. However, in a study comparing gizzard morphology for different avian herbivores, it was found that the increased quantity of grit in the gizzard interfered with the damaging effect of the translational movement on the contents, as less exerted force was distributed on the food, accordingly less damage was done on grass particles (Moore 1998c)

Moreover, Amat and Varo (2008) suggested an inverse relationship between Alkali-Bulrush tuber size (food) and the amount of grit ingested to optimize gizzard capacity by Graylag Geese. The authors suggested that food characteristics would influence grit function. For instance, the oval shape of the tuber will cause more empty spaces and unfilled gaps between

tubers as they accumulate and their size difference increases. As a result, a larger amount of grit will be required to process the larger tuber which is coarser than the smaller one.
A positive correlation was reported between quantity of coarse food consumed and quantity of grit ingested (Gionfriddo & Best 1999). However, and contrary to the previous statement, as the gap between tuber increases and more grit fills those gaps, food intake must be limited. Therefore, in order to optimize gizzard capacity and process, geese can adjust their grit intake and selectively eliminate the excess to consume more food instead.

Diet characteristics (hardness and coarseness) and food particles size predict grit particle size needed (Gionfriddo & Best 1999). For example, two studies conducted by Norris et al. (1975) on the Norwegian willow Ptarmigans and May and Braun (1973) on the white-tailed Ptarmigan showed that when birds were fed birch, twigs, leaves and buds, they consumed and excreted 2-4 times the amount of stones (of greater mean size) used by birds on pelleted diet. This was attributed to the coarseness of the food, which accelerated the disintegration of the grit and hence its excretion. In contrast, when softer food was given, less mechanical breakdown was required and consequently a decrease in grit mean size was detected. Moore (1998a) found that smaller grit resulted in greater grass breakdown when using an artificial geese gizzard compared to larger grit. The author ascribed this improvement to the larger surface area covered by the smaller grit and consequently, a larger number of sharp points contacted the grass and contributed to its breakdown.

In the second and third growth periods of this study, both groups achieved equal and consistent performance which demonstrates that grit did not hinder birds' performance, potentially for the aforementioned reasons. Although no digestibility analysis was carried out, FCR identical values could indicate that grit supplementation did not improve digestibility and hence the utilization of the pelleted feed. This is in accordance with the work of Sibbald and Gowe (1977) who observed no improvement in feed utilization and also found no adverse effect on livability. Accordingly a similar mortality rate was reported. The same observation was recorded in this study.

5.2. Particle Size Distribution at Different Ages:

Concurrent with this study's findings, Tagami (1974), as cited by (Gionfriddo & Best 1999) found that chickens retained more medium-sized (1.7-2.4mm) grit than large (2.4-3.4mm), and they retained very few small (0.6-1.7mm) particles.

In the current study, the significant increase in the proportion of the medium-sized (2.5, 2 and 1.6mm) grit when compared to the initial grit curve is likely due to the rapid abrasion of relatively larger particles caused by the abrasive grinding activity of the gizzard since they occupy larger volume and are particularly more prone to erosion due to frictional contact.

A significant increase from 3% to 12% and 11.8% at 10 and 21 days respectively of particles retained on the 1.6mm sieve was observed. The confounding factor is that larger particles may pass through the digestive tract and relatively increase the proportion of small particles. However, the proportion of particles retained on the 0.8mm sieve increased from 0.4% to 5.4% at 10 days. Since there were no particles (< 1.6mm) in the initial grit (given to birds), the increase in smaller particles proportion supports the contention that larger particles were considerably abraded and ground to a smaller size.

Besides the demonstrated erosion of larger particles (> 1.6mm), the increase in the proportion of small particles (< 1.6mm) may also be due to the tendency of smaller birds to selectively retain more of the small particles and use them to assist grinding.

Smith (1960) studied the influence of grit size and surface condition on digestibility of chicken and its retention in the gizzard. He noted that as grit size decreases, so does its retention time as opposed to larger grit and that regardless of the size and surface conditions, grit will assist the gizzard in the grinding activity as long as it is retained. The reduction in the proportion of the particles (retained on the 0.8mm sieve) at older ages (21 and 28 days) could also prove that smaller particles by this age were passed out and eliminated.

On the efficacy of small particles retained by younger birds (10 days) to assist grinding, Rowland and Hooge (1980) found that 6% sand can be added to broiler starter diet to improve feed efficiency. According to (Oluyemi et al. 1978), an improvement in food utilization was noted when (dietary fillers) sand or grit were added to turkey diets. In the same study, it was observed that sand passed out of the gizzard at a faster rate than grit and that both fillers did not accumulate in the gizzard, but were ground to a size no longer useful to assist in grinding and thus eliminated. In conclusion, the authors attributed the benefit of fine particles to their fast passage into the gizzard and its larger surface area in exposing food particles to enzymes even though they were of low value in grinding compared to grit.

Access to grit was facilitated by its availability to birds. A continuous consumption was observed and birds showed no preferences with regard to grit size as nearly all plates were emptied. Trost (1981) reported a reduction in gizzard grinding action when a soft diet is consumed, resulting in less wear for hard particles if inadvertently ingested. Similarly, Mangold and Felldin (1909) as cited by (Trost 1981) found that gizzard rhythm was much slower with soft food, and the harder the food the faster the contractions of the gizzard. Due to the hardness of the retained grit, a faster contraction and grinding action is therefore expected in the gizzard. Accordingly, grit of larger size (3.15 and 2.8mm) will grind on each other more frequently at a fast rate (due to the higher surface covered), speeding up the degree of erosion and increasing the proportion of smaller particles. In addition, Waite (1935) stated that granite grit has a tendency to shatter under the mechanical pressure of the gizzard and thus disappear rapidly.

Contrary to what was expected, erosion degree of grit in the gizzards had almost the same pattern, independent of age. This is graphically visible since at different ages, particle distribution followed the same trend and there was no difference between 10 and 21 days (uneaten grit from two pens was removed at 18 days), although at 28 days the proportion of particles (> 2.5mm) was higher and that of a smaller size (< 2mm) was numerically lower.
It could be suggested that there was a limit in the degree of erosion of grit in the gizzard during the experimental growth period. This observation is in partial accordance with Vance (1971) who speculated that grit in pheasants' gizzards will not undergo any further reduction in size (to assist in grinding or for mineral extraction) and will pass almost completely through the digestive tract. Instead, the bird will replenish its stock and selectively consume more grit or mineral-rich grit to fulfill its requirements. Vance (1971) supported his hypothesis on the basis that 25% of the grit fed was recovered from feces almost whole and intact. However, in the current study grit did not pass out to a high extent and it is less likely to be due to the need for mineral since the diet was balanced and broilers are not egg producing bird. However, due to the frictional erosion that reduced the sharpness of angles of the initial grit (see **Appendix**) it could be postulated that birds

may have consumed new stones, with sharp points and rough irregularities on their surface since they are more efficient in grinding as described by (Moore 1998a).

It was reported that grit availability and ingestion rate are the determining factors in grit elimination or retention (Gionfriddo & Best 1999). For instance, if chickens were deprived access to grit, consumed grit will be retained for a significant period of time (up to 1 year) in the gizzard and not pass through until gradually worn away and lost its value as a grinding agent (Keith et al. 1927; Scott & Heuser 1957). In contrast, if grit is plentiful and readily accessible, birds will consume and retain it briefly in the gizzard, before eliminating it in considerable amounts daily (Nestler 1946; Gionfriddo & Best 1995; Gionfriddo & Best 1999)

During dissection, an interesting observation that supports the previously mentioned authors' finding was recorded. Grit stones of different size distribution were found in the small intestine of several birds at different ages (10-28 days). Based on a subjective judgment, younger birds (10 days) had more stones that passed through the gizzard to the small intestine (second and third part) with larger portion of particles (> 2.5mm) compared to older birds (28 days), (Figure XX). This is consistent with Keith et al. (1927) and Scott and Heuser (1957) since birds with low or no access to grit (supplementation stopped at 18 days), will retain their consumed grit longer, and eliminate it at a slower rate (as shown by the difference in number of stones between birds at 10 and 28 days).

Additionally, there is a possibility that larger stones excreted at an earlier age could be recycled and reused by birds of the same age or older, as it is more likely to be perceived than smaller stones. Also, since particle size distribution is expressed in percentage, any change (decrease or increase) in any particle proportion will have the opposite effect on other fractions.
With longer retention time, the rate of abrasion increases and smaller particles that were eroded to a critical size would eventually pass out the digestive tract and disappear, consequently causing a relative increase in larger grit proportion (at 28 days).

Therefore, it is plausible to conclude that, the longer grit retention time in older birds (caused by the unavailability of grit), together with the higher portion of small particles being eliminated, and the larger stones excreted at 10 days of age are the explanation as to why larger particle portion was moderately higher among 28 day-old birds compared to 10 and 21 days.

5.3. Weight Distribution of 416 Gizzards at 30 days:

As discussed earlier (in the literature review section), pelleted feed or the lack of structural component in the diet will depress the development of the digestive organ and mainly the gizzard. In contrast, mash feed or coarser particles stimulated gizzard growth, increased its weight and consequently nutrient utilization was often improved. The increase in gizzard weight is attributed to the stimulatory effect of grit that necessitates extensive grinding activity for particle reduction due to its hardness and longer residence in the gizzard. Accordingly, birds will have a stronger and heavier gizzard with a more developed muscular wall and possibly an increase in its lumen size to allow additional feed intake and normal food passage rate.

Grit-fed birds developed significantly heavier gizzards than did the birds with no access to grit. This observation was anticipated since the presence of hard stones in the gizzard required further grinding activity for particle reduction, and correspondingly increased gizzard muscles' size. A pelleted diet alone decreased the additional grinding activity of the gizzard as there was no demand for it and resulted in smaller organs.

Gionfriddo and Best (1999) reported that domestic chicks fed grit, often developed larger and heavier gizzards than those with no access to grit. The result in this study is in accordance with Gionfriddo's statement and, as well, supports the findings of Riedel (1950), Kennard and Chamberlin (1959), Spencer and Jenkins (1963), Thomas et al. (1977), Jones and Taylor (1999) and Garipoglu et al. (2006), all of whom significant relationship between grit supplementation and gizzard development.

In contrast to the reported benefits of an enlarged gizzard, the considerable increase in gizzard size was not a determining factor for improvement in body weight or feed utilization since birds' performance in both treatments was equivalent. This conclusion is concurrent with Scott and Heuser (1957) work, who inferred that gizzard size or its increased relative weight were not conclusive criteria for an enhanced feed utilization. The authors found that hens with access to oyster shell with whole grains performed poorly in comparison with those supplemented with insoluble grit despite the fact that gizzards in both groups were equally stimulated and had enlarged muscle size. Those findings however, were not sufficient to solely conclude that gizzard size is not a criteria for better feed efficiency. Excess or imbalance in calcium intake (in the oyster shell) can have negative implications for the bird and a subsequent physiological impairment (Smith & Kabaija 1985).

In this study, the presence of grit in the gizzard evidently had no effect on overall pelleted-feed intake and thus neither altered feed particles passage rate nor feed retention time nor its contact with digestive juices. Therefore, we can speculate that nutrients digestibility was not improved. Svihus and Hetland (2001) speculated that nutrients surplus in the digestive tract (due to high feed intake) will cause lower nutrients digestibility as in the case of cold pelleted Norwegian-wheat based diet. However, this effect was reversed by reducing feed intake (changing diet to mash form), or by regulating feed flow in the gastrointestinal tract (addition of whole grain) or by diluting nutrients concentration with fibrous powder. A comparable hypothesis was tested by Sacranie et al. (2012) who observed an improvement in starch digestibility even after diluting a steam-pelleted diet with 15% oat and barley hulls. The authors suggested that the prolonged retention time in the gizzard is caused by the hard fibrous characteristics of the hulls that require longer grinding time and thus extended contact with enzymes and acid which accordingly increase the capacity of digestion and nutrient utilization. Another study that supports the previous hypothesis showed that, the accumulation of coarse materials or fiber particles in the gizzard will slow the passage time of fine particles and induce higher HCl and enzymes secretion (Mateos et al. 2012). Finally, Fritz (1937) specified that grit used as grinding agent, will work best if the feed is coarse and granular.

5.4. Grit Weight Distribution among Dissected Birds at Different Ages:

During each growth period, birds showed different characteristics in grit retention. Availability and access to grit increased the amount in gizzards, although some birds retained only few. When deprived of grit, the majority of birds had lower amounts. In addition, birds of the same age (within each period) appeared to have a natural variation in grit retention and elimination. This observation is in line with and Hollister (1991) who reported a great individuality between birds in the amount of grit retained in their gizzards. Likewise, Gionfriddo and Best (1999) stated that even within a species, amounts of grit found in gizzards varied greatly. It can be thus concluded that even with the high variation between birds, general performance was unaltered.

However, given the high natural variation between birds in eating and retaining grit, it was observed that, a higher mean grit weight in the gizzards appeared to slightly increase the variation in birds' body weight. Perhaps, it is due to the higher volume occupied by grit in some gizzards and subsequent low available space for food.

Despite the high CV= 77.1% in gizzard stone weight in birds at 28 days, their body weights did not vary as much (CV= 10.6%) compared to birds at 21 days (**Table 9**).

Dissected birds at 21 days had on average 4.83 gr of stones (230 stones) in their gizzard and correspondingly a CV=16.2% for body weights. We could therefore speculate that higher average stone weight in the gizzard (4.83 vs. 3.31 gr) in addition to the high variation in stone intake between birds, may have slowed down feed intake on an individual level (it was seen that 75% birds of the birds at 21 days had more than 4 gr of grit in their gizzards) and thus created lower uniformity in body weights (CV=16.2 vs. 10.6%). However, the 6% difference in weight variation is not a firm evidence that feed particles were obstructed by excess grit, and this was discussed earlier.

In order to determine the source of the high variation between birds, factors like type of feed, age of the bird, bird species, grit characteristics and housing conditions were kept constant and the only variable was the bird's gender. Knowing that the broilers were unsexed, it could be argued that the gender would affect intake and retention. For instance if sex was a determining factor, we would have seen different chart characteristics (a symmetrical distribution for example) or values concentrated in opposite sides marking dissimilarity between male and female. However, it is clear that sex at this immature physiological stage is not a determining factor, but rather it is the inevitable natural variability within the birds themselves that determines intake and retention.

5.5. Effect of Grit Amount on Empty Gizzard's Weight.

The increase in grit amount found in gizzards seemed to influence gizzards' empty weight as shown by the regression analysis at 10 and 21 days, however, the effect was not clear at the end of the trial (28 days). That is, the increase in grit amount may have caused a relative increase in the gizzard's holding capacity (due to additional stimulation) thus allowing the passage of food to continue normally, and provided enough space for additional food quantity. The lower relationship at older age (28 days) could be possibly due to the fact that a long period has passed since the birds had access to grit and that the grit percentage of the gizzard content in that period was lower (21% vs. 36%). This was described by (Starck 1999) as a reverse phenotypic response in the gizzard since a decrease in stimulation eventually reduces gizzard enlargement. For instance, Starck (1999) found that following 14 days of fiber inclusion (45%) in the diet of adult Japanese quail, an increase in gizzard size and mass were observed, and that the increase in mass was twice as much as the original size. However, after switching to normal diet (lower fiber inclusion) also for 14 days, a subsequent decline in gizzard size and weight were seen. Starck (1999) concluded that "there was a matching between load and capacity" as the gizzard responded phenotypically by either enlargement or reduction in size and mass.

5.6. Mean Grit number at Different Ages:

Several studies showed the quantity of grit retained among different types of birds and the variation within the same species. For instance, quantity varied from none to a thousand stones, and in some gizzards, grit quantity accounted for more than 60-70% of the total gizzard volume, where in others, it almost outweighed half of the content weight (Gionfriddo & Best 1999; Amat & Varo 2008). In the current study, grit accounted for almost 36% at 10 days and dropped to 21% at 28 days, as observed during dissection. It is obvious that companion birds, granivorous wild birds and waterfowl have a slower growth rate compared to the domesticated animal kept for production purposes. The majority of the birds reported in the literature are wild and their diet is influenced by the season, as is their grit intake and retention. Since they are not genetically selected for fast growth, a high grit volume or count will not impede their growth rate. In fact, it will assist the bird in food utilization (discussed earlier). In contrast, production birds, like broiler chickens are selected for fast growth and high feed intake and thus will benefit more if maximum space is allowed for food. As a consequence, a lower percentage of grit in the gizzard would be expected for birds with high feed intake requirements such as broiler chickens.

6. Conclusion:

Despite no observed improvement in providing insoluble grit to broiler chickens maintained on a pelleted diet, this study neither found any detrimental effect on general performance parameters, even though a wide variation and a high amount of grit were found in a considerable number of gizzards during all growth periods.

Gizzard's enlarged lumen volume as a result of increased muscular activity due to grit stimulation could be a contributing reason why grit-fed birds had the same feed intake and body weight as did the birds in the NGG.

Even with high grit intake variation, the gizzard's size and holding capacity seemed to increase proportionally with the amount of grit ingested at 10 and 21 but not at 28 days.

In addition to its hardness, the even distribution of grit among gizzard's bolus, suggests an efficacy in grinding since more surface area is allowed for the grit particles and thus more rubbing contact points with food and grit itself.

The significant amount of grit found in gizzards even 12 days after withdrawal denotes that either the quantity offered was higher than required, or withdrawal day had to be earlier than 11 days or that elimination rate was slow due to feed characteristics.

Further studies are necessary to evaluate the effect of different grit size ranges (large, medium, small) on pelleted feed utilization, or the inclusion of fibrous or coarse particles (oat hulls) before or following grit provision to speed up grit erosion. Reduction of grit quantity (half) and substituting it by whole grain (included in the pelleted die), and the effect of grit on gut health would be also worth investigating.

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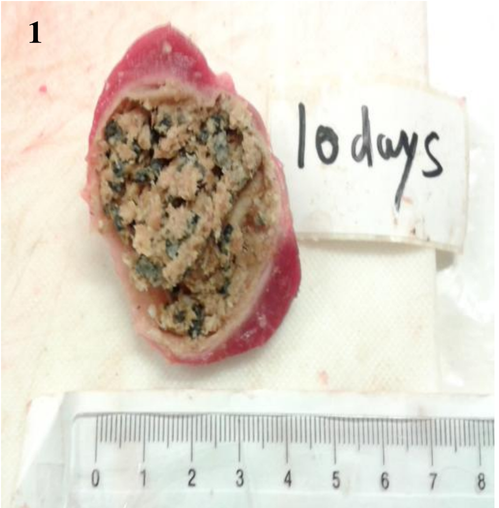
8. Appendix

Dissection photos (Photo credits: Itani 2014)

1


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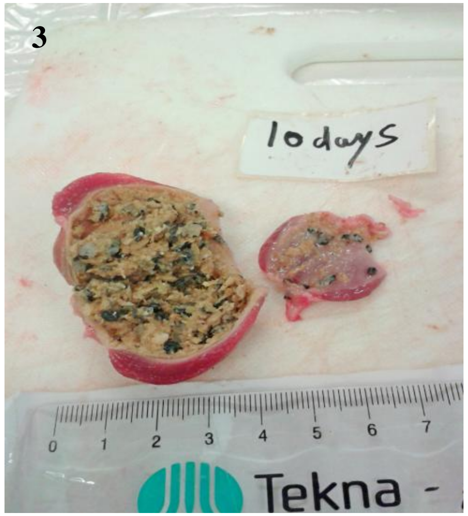
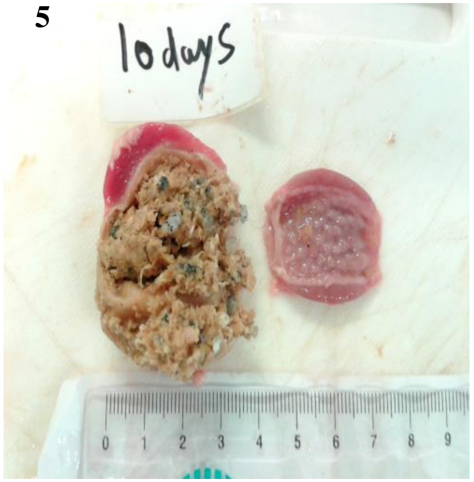


Grit seemed to be evenly distributed among the gizzard content.

4


5


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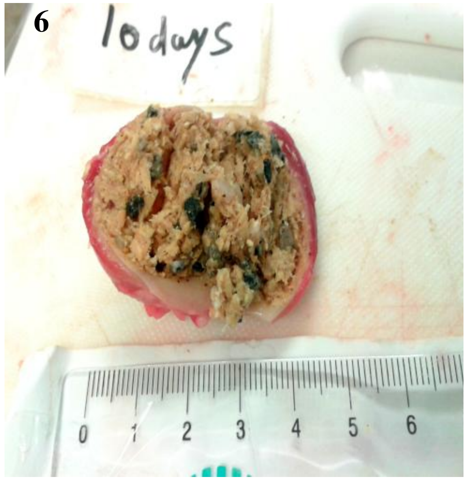
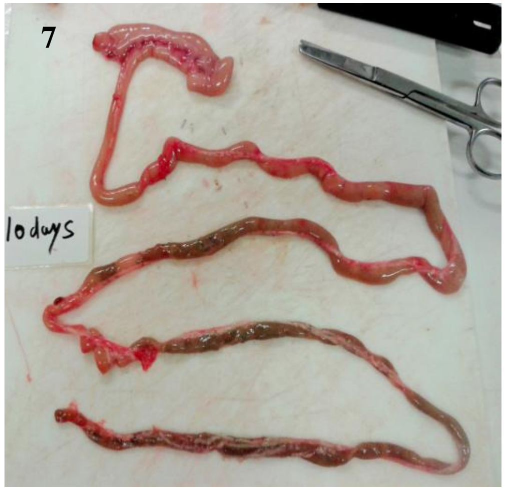
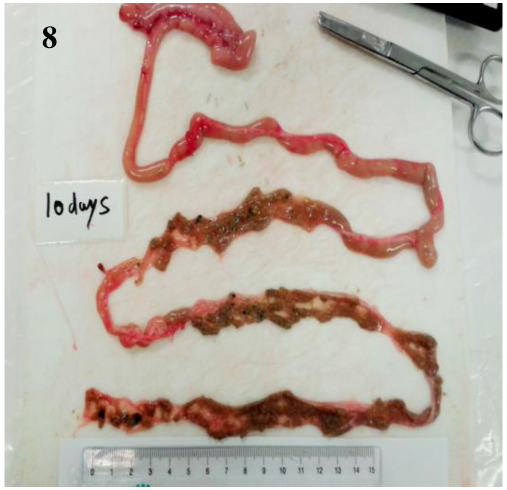
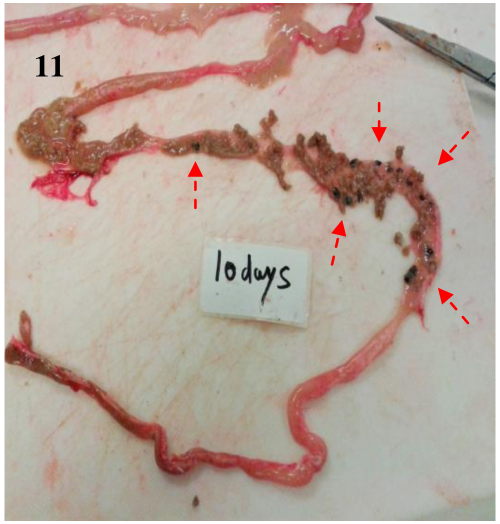
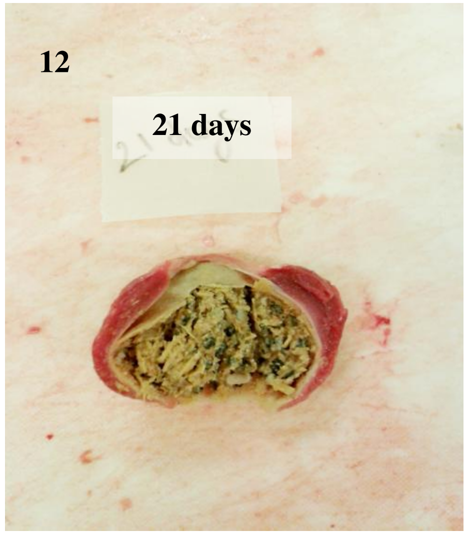


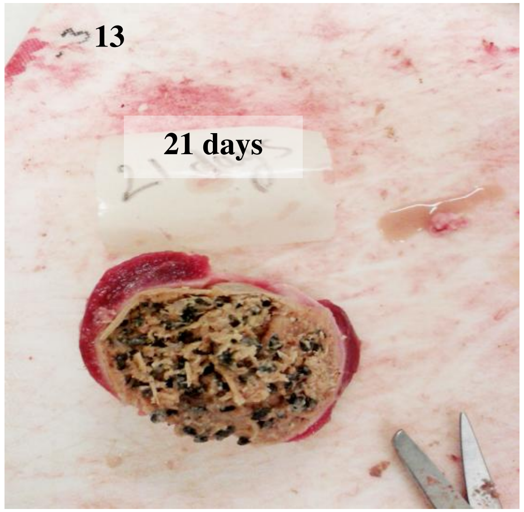


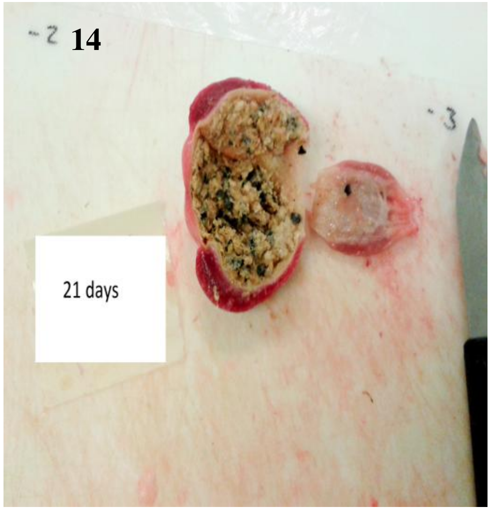


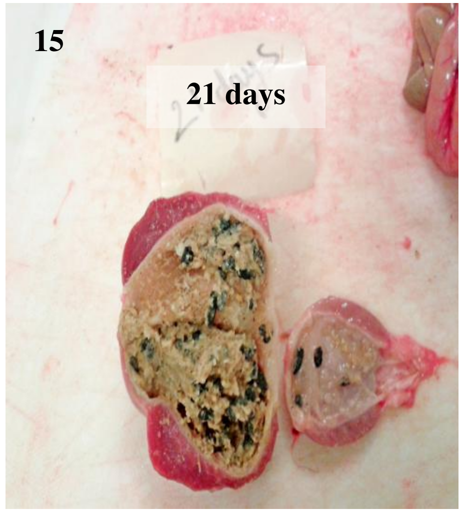
Considerable amount of grit (of different size range) passing out the GIT at 10 days



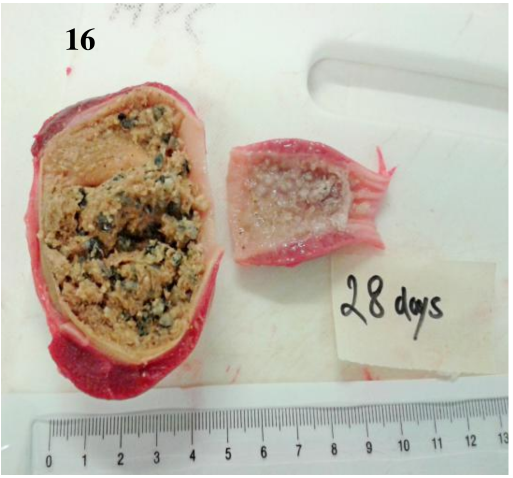


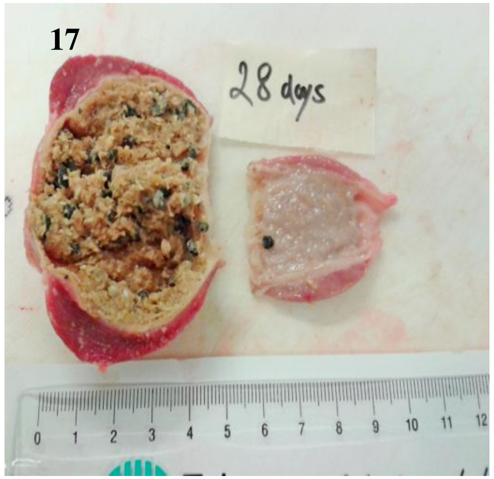


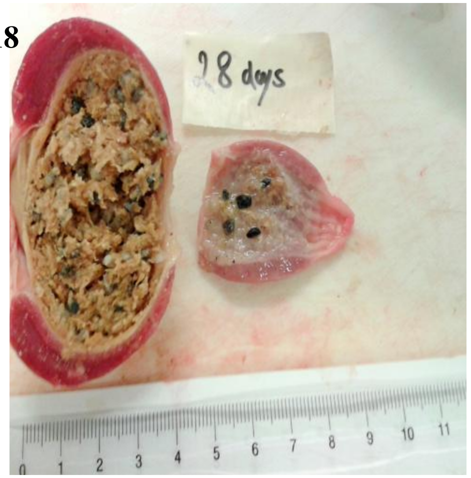


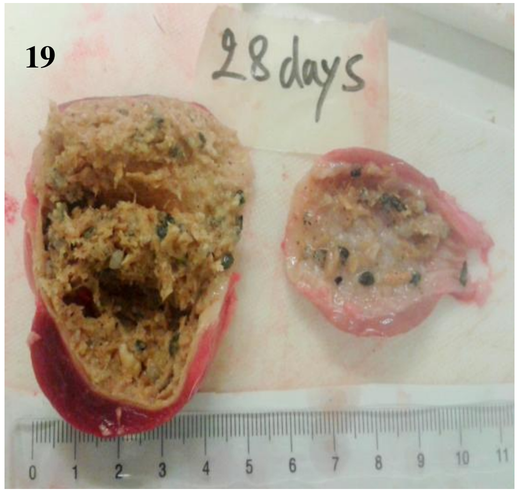


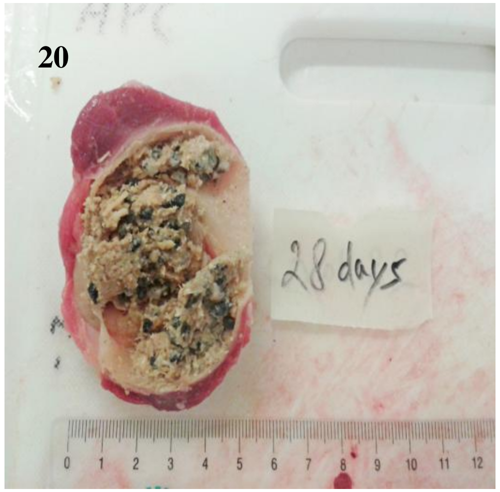
Some grit was seen in the proventriculus 3 days after withdrawal (possibly due to digesta reflux)



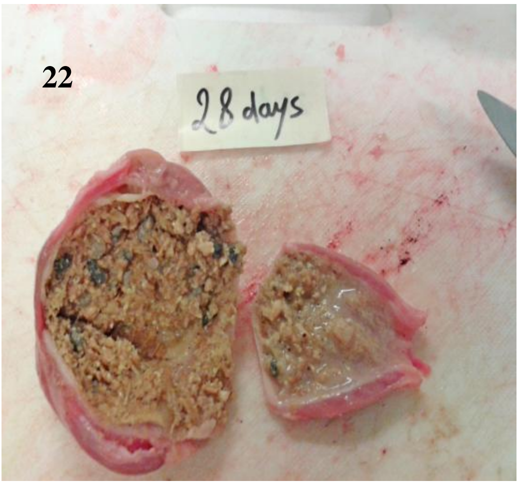


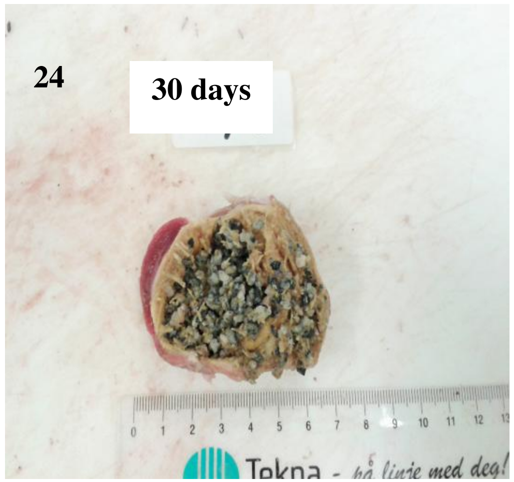




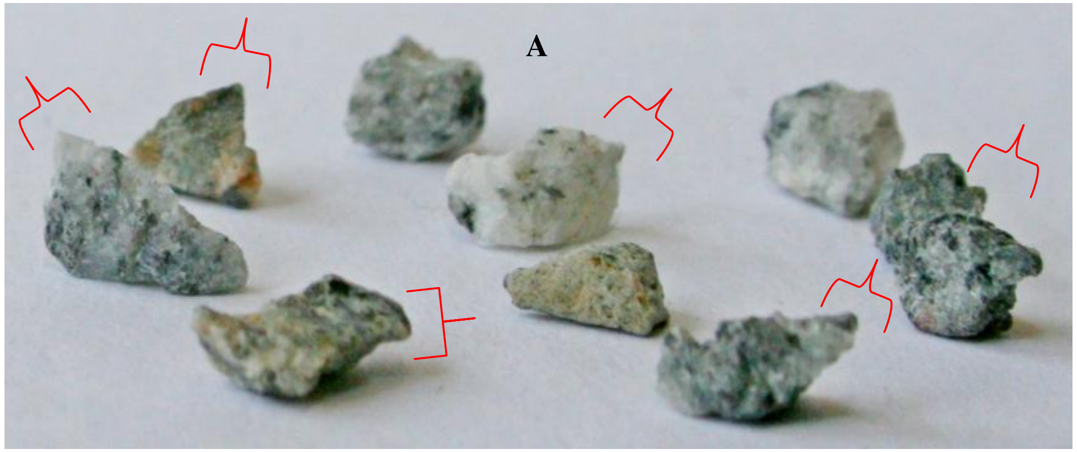


A significant amount of grit in some gizzards 12 days after withdrawal. Grit output was very low at older age (fig. 21)

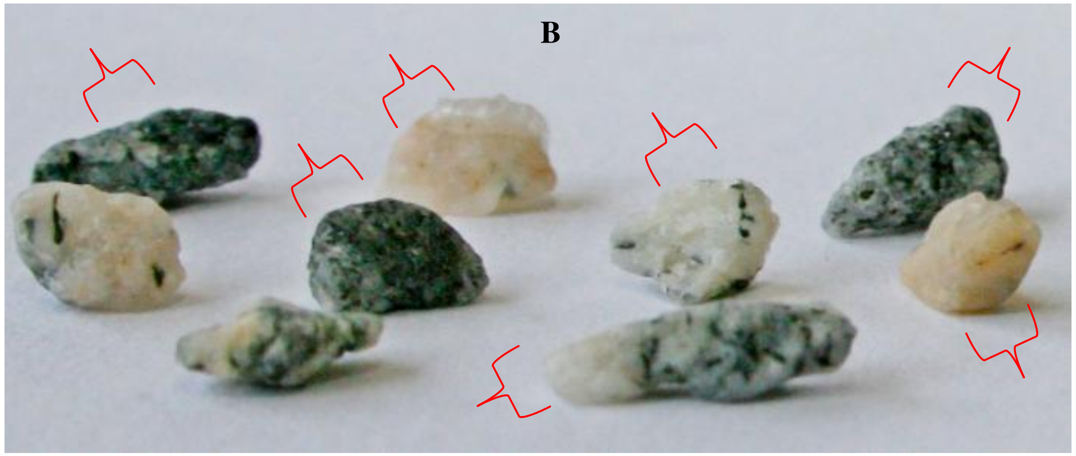




A: **As-fed grit**: rough surfaces with irregularities, sharp-edged and pointed ends.



B: **Grit from gizzards: 30 days old birds**
Blunt and smooth surfaces, round edges, due to the abrasion in the gizzard.



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