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The effect of grinding method and extent of pelleting of broiler diets on performance, feeding behavior, and digestive tract functionality

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Abstract

Two experiments were conducted for the accomplishment of this thesis. The grinding experiment was carried out with several normal screens and novel screens to study the effect on particle sizes. Grinding with novel screens was satisfactory for the coarser grinding. Based on the results of the grinding experiments, a 3 mm normal screen and two novel screens were selected for the production of wheat and maize-based seven experimental diets. Broiler experiment was carried out with the hypothesis that it would be possible to increase the microstructure for proper gizzard development and at the same time macrostructure to assure the feed intake is not compromised. Three diets were pelleted (SP, VCP, and MCP) from each grinding level and the remaining four heterogeneous diets (VCPM1, VCPM2, MCPM1, and MCPM2) were the mixture of coarse cereals with the pellets. Ground cereals were initially sieved at two distinct levels (one and two sievings) for heterogeneous diets, and the resultant fines were then subjected to pellet production while coarse particles were then blended with pellets afterward. Particle size distribution of the pelleted-only diets showed that the percentage of coarse particles > 1 mm decreased after pelleting compared to the mash particle size. Feeding pelleted-only diets did not affect feed intake and weight gain. There was no consistent effect of heterogenous diets on feed intake and body weight gain, however, the tendency was lower for heterogeneous diets. None of the dietary treatments had an impact on FCR ($P > 0.05$). Relative gizzard and content weight increased ($P < 0.05$) with the inclusion of the heterogenous diets compared to the standard pelleted diet. The starch and protein digestibility between the treatments did not differ significantly ($P > 0.05$). In comparison to the moderately coarse cereals mixed with pellets after one sieving, the standard pelleted diet had a larger percentage of litter in the gizzard. Feeding behavior showed that birds spent more time eating diets MCPM1 and MCPM2 compared to the MCP. Feed preference trial showed that big particles > 2.8 mm disappeared first showing birds' preference for pellets when offered heterogeneous diets. When represented as D10, D50, and D90, the intestinal particle size determined by laser diffraction showed no significant change ($P > 0.05$) in any of the small intestinal segments. More than 50% of the particles in the duodenum are smaller than 0.1 to 0.2 mm, while 50 % of the particles in the jejunum and ileum are below 0.2 to 0.5 mm (200 µm to 500 µm) when expressed as a volume percentage. In both VCP and VCPM2-fed birds, excreta examination revealed that around 10% of the particles were larger than 1 mm.

Overall, this experiment indicates that coarser grinding will result in less energy consumption and higher grinding capacity. Poor feed intake and weight gain were observed when fed heterogenous diets while a positive response was observed in terms of gizzard development and particle size distribution in the intestinal contents indicating that there is sufficient space for increasing both micro- and macrostructure in broiler diets. Therefore, diet selection can be a major limiting factor to broiler performance.

Key words: grinding; screens; macrostructure; microstructure; heterogeneous diets

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Sachin Dhakal

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This thesis is dedicated to my late father Mr. Dolraj Dhakal. May his soul rest in peace.

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Abbreviations

AI	Anterior Ileum
AJ	Anterior Jejunum
BWG	Body Weight Gain
CC	Coarse Corn
DM	Dry Matter
FCR	Feed Conversion Ratio
FI	Feed Intake
GIT	Gastro-Intestinal Tract
GMD	Geometric Mean Diameter
HCL	Hydrochloric Acid
HM	Hammer Mill
MC	Moderately Coarse
MCP	Moderately Coarse and Pelleted
MCPM1	Moderately Coarse cereal Mixed with Pellets after one (1) sieving
MCPM2	Moderately Coarse cereal Mixed with Pellets after two (2) sieving
NR	Not reported
PDI	Pellet Durability Index
PI	Posterior Ileum
PJ	Posterior Jejunum
PSD	Particle Size Distribution
SCFAs	Short Chain Fatty Acids
SP	Standard Pelleted

TiO_2	Titanium Dioxide
UV	Ultraviolet
VC	Very Coarse
VCP	Very Coarsely grounded and Pelleted
VCPM1	Very Coarse cereal Mixed with Pellets after one (1) sieving
VCPM2	Very Coarse cereal Mixed with Pellets after two (2) sieving
WW	Whole Wheat

Units used

d.	days
hr.	hours
Kg	Kilogram
M	Mole
MJ	Megajoule
°C	Degree celsius
mg	milligram
ml	milliliter
mm	millimeter
µm	micrometer

1. Introduction

The world population is expected to reach more than nine billion people by 2050. With 70% more food production requirements, food security will become a key challenge, necessitating the utilization of finite natural resources such as water and arable land, as well as adaptation to predicted climate change (FAO, 2020). It is critical to ensure that the food production system, particularly the poultry industry, is sustainable. The goal of broiler production is to produce meat and meat products that can be consumed. Broiler production accounts for a large component of poultry production, with global yearly production expected to reach 105.26 million metric tons in 2023, with a predicted growth rate of 1.73 percent from 2019 to 2023 (Maharjan et al., 2021). Modern broilers are high-yielding and fast-growing, with the highest feed efficiency ever recorded in broiler evolution (Siegel, 2014). Based on species, poultry feed accounted for the highest global feed production volume. The 2021 Alltech Global Feed Survey predicts that the production of broiler chicken feed increased by 1% from 332.5 million tons in 2019 to 334.5 million tons in 2020 (Table 1). Broiler feed efficiency means less environmental emissions and reduced reliance on plant-based sources, lowering the carbon footprint per unit of production compared with other animals. Focusing on improving feed efficiency can provide a firm foundation for environmentally and economically sustainable poultry (broiler) production (Willems et al., 2013a). Because of genetic advancements made between 1994 and 2018, there has been a 16 percent reduction in energy consumption in broiler production, as well as a 15 percent reduction in greenhouse gas emissions (Aviagen, 2020). Genetic improvement and nutritional advancements are the driving forces for today's development in broiler production.

Table 1. World poultry feed production statistics (Alltech Global Feed Survey 2021)

World Poultry Feed Production (Million tons)

Region	POULTRY (Broilers) 2019	2020	Growth	POULTRY (Layers) 2019	2020	Growth
Africa	11.56	11.17	-3%	7.98	7.85	-2%
Asia-Pacific	141.18	143.6	2%	75.06	77.92	4%
Europe	55.12	54.87	0%	32.43	31.12	-4%
Latin America	60.99	62.04	2%	24.44	23.1	-5%
Middle East	8.17	8.11	-1%	4.57	4.42	-3%
North America	51.78	50.9	-2%	15.54	15.12	-3%
Oceania	3.79	3.89	3%	0.93	0.95	2%
TOTAL	**332.57**	**334.57**	1%	**160.96**	**160.48**	0%

Source: 2021 Global Feed Survey
**All numbers are in million metric tons.*

Retrieved from: Alltech. n.d. https://one.alltech.com/2021-global-feed-survey/.

Feed is the most expensive component of broiler production, accounting for up to 70% of total poultry production costs (Abdollahi et al., 2013b; Ravindran, 2013; Willems et al., 2013b). Feed processing, such as grinding, pelleting, or expanding, results in changes in the structure of the feed (Svihus, 2006), which is defined by particle size and distribution, as well as the physical form of the diet (Röhe et al., 2014). Although grinding incurs a significant expense in terms of energy consumption and feed mill capacity, grains for poultry are often finely processed in a hammer mill with a screen size of 3 to 4.5 mm. Fine grinding was considered a key to achieving high proper feed utilization in the poultry digestive system (Svihus, Kløvstad, et al., 2004), however, much literature emphasizes the importance of coarse particles in poultry diets (Svihus, 2011). It is not only ingredient composition and nutrient content that matters for good performance in poultry. The form and structure of the poultry diet are also equally important. It became evident that pelleting the feed to form a pellet was advantageous. In addition to boosting the feed intake, it decreases feed wastage as a result of fewer particles falling from the beak onto the floor (Behnke, 1994, 2001; Jensen, 2000). While grinding is done to make the feed's particle size smaller, pelleting is done to make the feed's particle size larger. So, in a pelleted feed, structure refers to both the structure of the pellets themselves and the particle distribution of the elements that make up the pellet. Thus, it is appropriate to refer to the structure of the pellets themselves as “macrostructure” and the structure of the particles inside of them as “microstructure”. As will be discussed in detail below, the macrostructure will only have an impact on feed intake pattern and total feed intake as pellets typically break down relatively quickly once they become moist in the upper digestive tract. On the other hand, the microstructure has a profound effect on digestive tract functionality, primarily through gizzard stimulating properties. (Ege et al., 2019; Svihus, 2006).

Routinely, types of equipment are used to grind up cereal grains into smaller particles before being utilized as feed for chickens. Cereals are typically ground by either passing them through a hammer mill or a roller mill to reduce their particle size. Simply making sure that the feed ingredients are not too finely ground will ensure that there are enough big particles in the diet. Cereals make up the majority of the diet and will have the biggest impact on the microstructure of the diet. Coarser grinding can be achieved by increasing the hammer mill screen opening diameter of the holes. Also, a coarser grind would boost grinding efficiency and use less energy. Reece et al. (1986) found that switching the 4.76 mm screen size of a hammer mill to 6.35 mm reduced energy consumption by 27%. To maximize production efficiency while minimizing the energy required for grinding, the relationship between feed particle size and broiler performance, gut health, pellet quality, and production efficiency must be considered. The relationship between hammer mill screen and particle size is discussed more below in the literature review section.

Since birds lack teeth, the mechanical grinding of ingested feed particles takes place in the gizzard, a special organ containing muscles that tightly contract to reduce the size of food particles (Svihus, 2014). Due to the increased necessity for particle size reduction caused by the inclusion of large cereal particles, the gizzard enlarges (Svihus, 2011). For diets based on maize and wheat, the grinding must be sufficiently coarse for the grain particles to encourage gizzard development. Svihus, Kløvstad, et al. (2004) found that the particles larger than 1 mm increased from 25% to 37% when wheat was ground from 3 mm to 6 mm, respectively resulting in a larger gizzard size. With maize diets, Parsons et al. (2006) discovered comparable outcomes. When maize was ground with an increasing screen opening of the hammer from 3.18 to 6.35 mm, the amount of particles larger than 1.18 mm rose from 38 to 55 percent, and the performance of broiler chickens was comparable. So, the advantages of improving microstructure are mostly related to the positive impacts on the efficiency of the digestive system, mainly the gizzard.

Particle size reduction is a requirement for digestion since it increases surface area and makes it easier for digestive enzymes to reach nutrients. The anatomy and function of the gastrointestinal tract may be affected by feed production and processing techniques. The gastrointestinal tract development, in particular the gizzard, adjusts quickly to the changes in the diet composition. Structural components such as whole or coarsely ground cereals, wood shavings, and oat hulls when included in the poultry feed stimulate the gizzard development and subsequent activities (Hetland et al., 2004). This is because a well-developed gizzard can grind feed particles more thoroughly, promoting enzyme secretion, thus increasing nutrient digestibility. Broilers fed coarsely ground mash diets or structural elements like wood shavings or oat hulls had better digestion, especially in terms of starch digestibility (Svihus & Hetland, 2001; Svihus et al., 2010). Naderinejad et al. (2016) demonstrated that coarse maize grinding improves nutrient and energy utilization in broilers given pelleted diets due to improved gizzard development and functionality. Also, the transit rate of digesta may be reduced by feeding coarse particles, which may promote enzymatic digestion and nutritional digestibility. The latter effects could be explained by the gizzard's ability to control the intestine transit time when coarse ground feeds are used (Carré, 2000). However, the feed materials flow more rapidly into the small intestine in birds with an underdeveloped gizzard, resulting in a large amount of particles (Hetland et al., 2002) and undigested nutrients (for example, starch) in the ileum (Itani & Svihus, 2019; Sacranie et al., 2017).

There are few data on how the feed's particle size affects particle size distribution in the intestinal contents. With the well-developed gizzard, it is expected to have very fine particles entering the duodenum after grinding in the gizzard thoroughly. Regardless of the feed's initial particle size, the gizzard has the amazing capacity to grind all organic components to a consistently fine size, according to Hetland et al. (2004). Regardless of the original feed structure, the digesta passing through the gizzard showed a similar particle size distribution, with the majority of particles being smaller than 40 µm in size (Hetland et al., 2002). In contrast, Lentle (2005) hypothesized that as the fraction of coarser particles in the diet increases, more of the coarser particles would pass through the gizzard, but that this would increase the efficiency of digestion because the digesta would be more permeable to the digestive enzymes. This hypothesis was based on observations that digesta comprises a mixture of large and small particles. In a study (Amerah, Lentle, et al., 2007), it was observed that birds fed mash diets had larger gizzards, and those fed pelleted diets had a higher proportion of big particles (1000–2000 µm) in their duodenal digesta. This indicates that there might be room for making diets with a coarser microstructure which will be beneficial from both nutritional and technical points of view. Nutritionally, coarser particles stimulate gizzard activity more effectively than fine particles and the technical part can be viewed as coarser grinding increases grinding capacity and lower energy consumption.

Feeding pelleted diets is a common practice today all over the world. In the first half of the 20th century, using chicken diets in mash (unpelleted) form was the standard feeding approach; however, in more recent years, using poultry diets in the form of pellets has taken precedence (Svihus, 2011). The highest feed intake can be obtained by pelleting the mash diets to produce the macrostructure, pellets, even if coarser particles in the mash diets will result in a higher feed intake than finer grinding. Feed intake (FI) is the main cause of body weight gain (BWG), and pelleting broiler diets are done primarily to boost FI (Engberg et al., 2002; Svihus, Kløvstad, et al., 2004). According to Abdollahi et al. (2011), pelleting during the starting phase (1–21 days of age) resulted in a 14% rise in FI of broilers. Pelleting improves feed efficiency in part because less feed energy is required for maintenance. Therefore, pelleting may result in increased productive energy (Nir, Twina, et al., 1994) as chicken-fed pellets spend less time eating compared to the mash diet (Reddy et al., 1961). Reddy et al. (1962) also demonstrated that the pelleted feed produced roughly 30% more productive energy than the mash. The process of pelleting begins with the addition of water in the form of steam in the conditioner and is completed with the extraction of the pellet using a die and roll in the pellet press. The literature review chapter will go through the impact of thermal processing (pelleting) on feed intake and broiler performance. The quality of the pellets is a crucial component of the chicken feed. Good quality pellets with fewer fines resulted in better broiler performance (Proudfoot & Sefton, 1978). In the feeder, there will always be particles smaller than the pellet because pellets tend to break during handling and transport. To assess how well the pellet can sustain pressure during handling and transportation, a durability test is conducted. This can be analyzed by the Holmen durability test and measures the percentage of intact pellets remaining after the Holmen test. Therefore, broiler performance is significantly impacted by the fraction of coarse particles in mash diets and the proportion of intact pellets in pelleted diets.

Birds can select different-sized feed particles from an early age, and as they get older, they tend to prefer larger particles (Nir, Shefet, et al., 1994). Traditionally, all the ingredients in poultry feed have been ground to the same general fineness. This was important when the meal is offered in mash form to prevent the birds from selectively ingesting items with high particle sizes (Reece et al., 1986). The size and kind of food to be eaten are determined by the shape and structure of the beak, age of the birds, development of the digestive system, etc. therefore the granulometry of the particle is crucial for controlling consumption (Neves et al., 2014). Measurement of feed particle size is known as granulometry. The uniformity of feed particles is thought to be significant for good performance for broiler given mash ration, as suggested by Nir, Hillel, et al. (1994) since the birds spend less time finding and picking the larger particles. Following an overnight feed deprivation, the first two hours of refeeding showed a relationship between feed consumption and grain size, with medium and coarse grains being more readily ingested by 7-day-old chicks (Nir, Hillel, et al., 1994). According to Douglas et al. (1990); Nir et al. (1995); Nir, Hillel, et al. (1994), chicks prefer particles between 700 and 900 μm. However, Amerah, Ravindran, et al. (2007a) found that chicks prefer particles between 600 and 900 μm while Portella et al. (1988) propose a particle larger than 1180 μm and for adult birds greater than 2360 μm. Birds' preference for different particle sizes will be discussed more in the literature review below.

The relationship between microstructure and macrostructure has effects all the way from the production stage to the broiler performance. It has been found that feeding poultry mash diets in contrast to feeding the pelleted diets, has a favorable influence on gizzard development (Amerah, Ravindran, et al., 2007a). This is due to pelleting's ability to reduce the size of the microstructure that makes up the pellets and almost eliminate the coarse microstructure (Abdollahi et al., 2013b), due to the narrow distance between rollers and die (Svihus, Kløvstad, et al., 2004). When feed materials, mainly coarse particles, are compressed between the rollers and the die to force the materials into the holes, the pellet press functions as a grinder (secondary grinding). As a result of varying levels of grinding, the pellet press will tend to smooth out variations in particle size and, consequently, microstructure. Svihus, Kløvstad, et al. (2004) wet sieved diets before and after pelleting and found that as a result of pelleting, the proportion of coarse particles decreased while the amount of fine particles increased. Before pelleting, hammer-milled wheat-based broiler mash diets had 40 to 50 percent particles smaller than 0.2 mm, which increased to 50 to 60 percent after pelleting in this study. Abdollahi et al. (2011) found that passing diets through the pellet die reduced the proportion of coarse particles above 2 mm and increased the proportion of tiny particles less than 0.075 mm, which is in concordance. These findings corroborate those of Engberg et al. (2002), who found that pelleting significantly reduced feed particle size and balanced discrepancies between coarsely and finely ground pellets.

Taking the aforementioned considerations into account, the conflicting issue between the feed intake preferences for larger particles and the nutritional and health benefits of coarser particles must be overcome. It is repeatedly mentioned that coarser grindings result in better gizzard functioning so preservation of coarse particles is of crucial importance in broilers' diets. One way to preserve coarse particles without compromising the pellet quality can be achieved by the coarsest grinding on the hammer mill (HM-9), and adjusting the largest roller-die gap (Vukmirović et al., 2017), increasing the pellet diameter, and/or not introducing the coarser particles into the pellet press. By sieving the hammer-ground cereals in a suitable sieve, separating the coarse and fines, and allowing only the fines to pass into the pellet press, it is possible to save the coarse cereal fraction. Then, mixing the coarse structure with thus obtained pellets would help to maintain the coarse structure in the finished diets.

The effect of mixing coarser particles and the pellets on broiler performance has not been documented more in the literature. Thus, there appears to be room for increasing structure (mixing of coarse particles bypassing pellet press and the pellets) in broiler diets, though further research is required in this area. This thesis is focused on two different parts, test grindings and animal experiments. The first aim was to select an appropriate hammer screen diameter for coarser grinding to be used in the production of unique experimental diets. Secondly, this thesis investigates the methods that could be used to maintain thus obtained coarse structure during the pelleting process and exploit the beneficial effects of coarser microstructure. The latter was investigated by letting the coarsest particles from the grinding process bypass the pellet press. The hypothesis was that by mixing coarse particles and pellets in the diet, it would be possible to maintain a high feed intake and that the microstructure of the feed would encourage the development of the gizzard and other functionalities.

1.1 Literature review

1.1.1 Feed processing process

Feed processing is the process of treating a feed before it is consumed by animals. A single step, such as grinding, or a series of processes, such as grinding, mixing, conditioning and pelleting, and cooling, can be used in the processing (Maier & Bakker-Arkema, 1992) as shown in figure 1 and are explained later in this chapter. Almost all of the feeds used in commercial poultry production are processed in some way. Most of the feeds are ground in a hammer mill and then pressed into pellets in a pellet press after it has been heated. Processing has two key effects: a) changes in the micro-and macrostructure of the feed; and b) heat-induced chemical alterations in some feed components (Svihus, 2006). The macrostructure is said to influence the feed intake while the microstructure influences gizzard development, functioning, pH, and health of the gastrointestinal tract (GIT) in chickens (Röhe et al., 2014; Svihus, 2006).

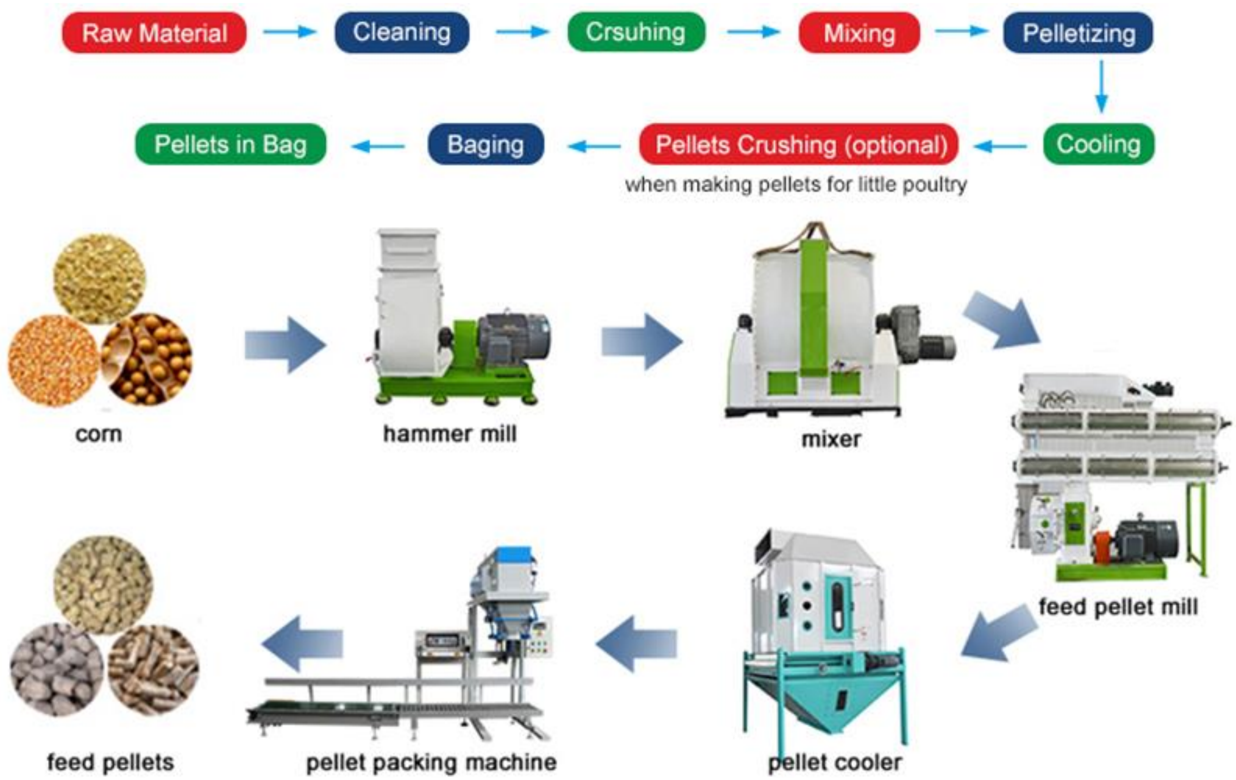

Figure 1. Animal pellet feed production line (Post-batch grinding)

Retrieved from: https://www.feedpelletmills.com/

1.1.2 Particle size reduction in the grinding process

The poultry industry has become more interested in feed particle size in recent years as it looks for ways to improve feed utilization and production efficiency. Smaller feed particle size contributes to better mixing efficiency and consistency, lessens ingredient segregation during subsequent processing, and enhances the quality of final pellets from a manufacturing standpoint (Behnke, 1996; Koch, 1996). Smaller particle size improves the relative surface area, which enhances digestibility since fine particles are more readily exposed to the digestive enzymes. However, fine grinding increases manufacturing costs, and underdevelopment of the gizzard occurs. As nutritionists explore ways to optimize feed consumption and production efficiency in poultry, interest in the impacts of feed particle size, feed shape, interactions between particle size and major cereal, and energy density has grown in recent years (Ege et al., 2019). The aforementioned factors can be taken into account by managing the various elements of grinding mills, including the choice of suitable hammer mill screen opening, and the choice of raw materials.

Grinding is an obligatory step in the feed manufacturing process for reducing the particle size of feed ingredients, and it can be done with a variety of mills. Roller mills and hammer mills are the most often utilized mills in the manufacturing of the animal feed industry (Rojas & Stein, 2017). Because cereal grains and pulse crops are rarely ground before entering the feed mill, these materials must be ground (Rojas & Stein, 2017). There are varied preferences for using roller mills or hammer mills in the feed sector. These choices are frequently based on the amount of grinding capacity required, the efficiency of the power utilized, and the sorts of feedstuffs employed (Rojas & Stein, 2017). The most important principles of particle size reduction include impact, compression, attrition, and cutting. Impact refers to material breakage induced by a high-speed collision between a particle and a hard surface (hammer mill), whereas compression refers to material breakage caused by compression between two hard surfaces (roller mill).

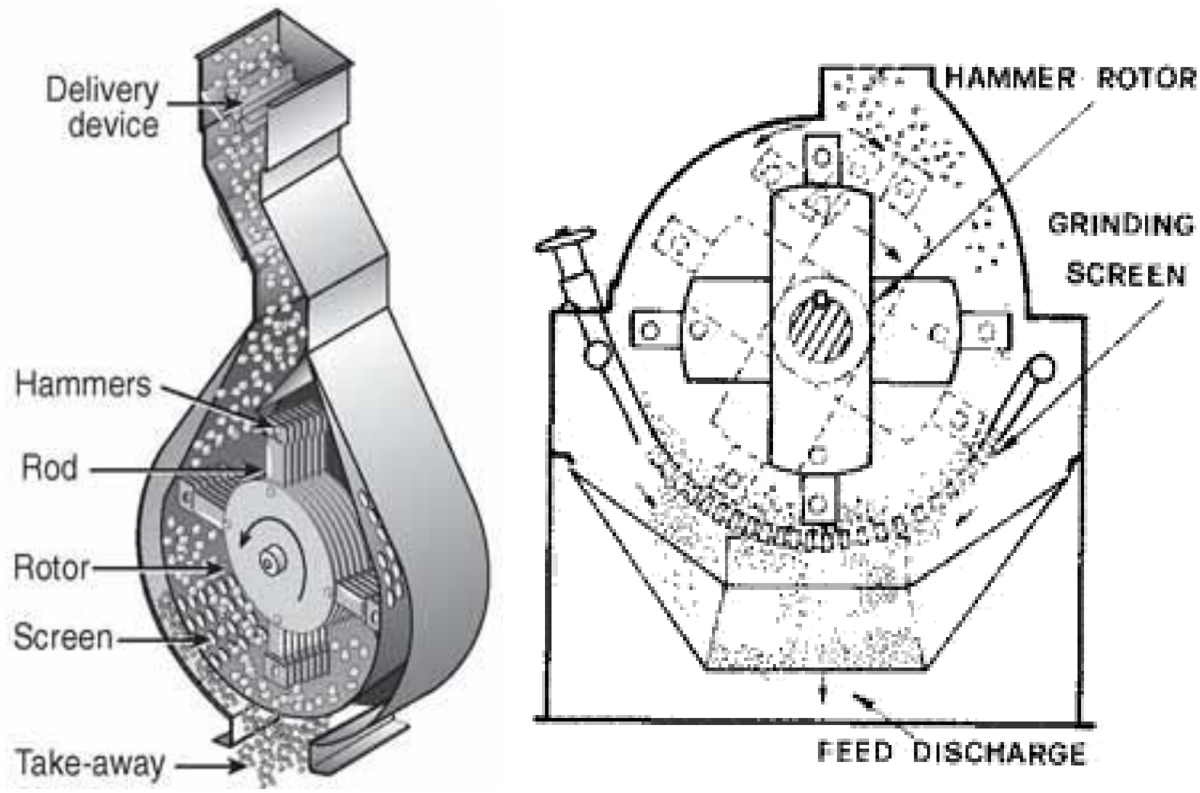

Figure 2. A typical hammermill

A set of hammers are suspended from a central shaft and housed within a robust metal casing in a hammer mill as shown in figure 2. Except for the top aperture for the feeder, the hammer mills' perimeter is entirely covered by screens. Two screens are often present, one on either side of the circular. The screen has holes throughout it, and the percentage of the open area created by the holes is crucial for the feed materials to pass through the screen. 40 percent of the space is typically open, however, this percentage might range from 30 to 50 percent. Depending on the requirement of the animals, the diameter of the holes in the screen varies from 3 to 9 mm. The impaction/attrition between the quickly moving hammers and the relatively slow-moving particles causes particle size reduction in a hammer mill. Appropriately sized materials can pass through a screen in the milling chamber as a finished product (Lyu et al., 2020).

When selecting the best mill, the pros and downsides must be evaluated. Hammer mills offer a higher grinding capacity, and changing the screen makes switching from one grain to another much easier. Hammer mills, on the other hand, produce more fine particles and dust and use more energy per ton of material than roller mills. The grinding procedure and mill type have a direct impact on particle size distribution. The best particle size distribution of feed obtained during the grinding process may be directly influenced by the optimal condition of the hammers in a hammer mill. The size distribution of hammer mill particles ranges greatly from the geometric mean, with some large and many small particles (Svihus, Kløvstad, et al., 2004). The benefits of grinding/reducing the particle size of components for feed processing and animals are numerous. According to Goodband et al. (1995), reducing raw material particle size improves mixing characteristics by reducing segregation with other ingredients in the mixture, as well as pelleting capacity and quality. Grinding also improves the surface area accessible for digestive enzymes to interact (Mavromichalis et al., 2000). Mixing got more complicated as more components were available, as well as increasing information about nutrient requirements and composition in the feed. Grinding became even more necessary as a result of this, as grinding improves the mixability of substances. The pellet quality has been a concern with coarsely ground grains in the diet. Because of the lower particle surface area and weak spots in the pellet, as the number of coarse particles in the pellet increases, more fractures may occur. Although Svihus, Kløvstad, et al. (2004) found a slight drop in pellet durability with coarser grinding, other studies have found that coarser grinding improves pellet quality (Behnke, 2001; Reece et al., 1986; Vukmirović et al., 2017). Thus, the reason for grinding can be summed up as follows:

- improving digestion by increasing the surface area
- increasing surface area and hence boosting particle binding ability in pellets
- improve ingredient mixability
- increasing particle homogeneity

1.1.3 Effect of hammer mill screen opening (HMSO) on particle size

The most important factors affecting particle sizes in the grinding process are raw materials, equipment factors, and grinding adjustment factors. The different cereals behave differently in the grinding process because of their size, moisture content, fiber contents, degree of hardness, etc. The hard wheat diet had 32.7 percent of particles larger than 1 mm, compared to 18.7 percent in the soft wheat diet, when passed through the same screen size in the same hammer mill (Amerah et al., 2009). The size of the cereals also affects grinding results as small seeds may pass through the hammer mill screen unbroken. Another factor responsible for the grinding capacity difference is the screen diameter of the hammer mill. Production capacity was decreased and energy requirements for the grinding process increased as particle size was reduced where milling corn to 400 µm consumes three times as much electrical energy as milling corn to 1,000 µm (Wondra et al., 1995). Also, the effect of screen hole diameter on particle size distribution is another equipment factor responsible for the difference in grinding results difference, as mentioned before. In general, having a big hole diameter is beneficial because it makes it simpler for ground cereals to flow through, which reduces dust and improves grinding efficiency in terms of capacity and energy used per tonne ground. The increased opening diameter of the hammer mill screen resulted in a significant ($p < 0.001$) reduction in the mill's specific energy consumption. In terms of treatment, the specific energy consumption of the 3 mm hammer screen (2.24 kWh/t) was significantly higher than the 6 mm and the 9 mm hammer screen (0.64 kWh/t and 0.41 kWh/t, respectively) (Vukmirović et al., 2017). Screen perforation has a significant impact on the grinding outcome. As was previously said, increasing the diameter of the screen hole increases grinding capacity. This is due to the increased amount of coarse particles and fewer fine particles (Table 2). The percentage of open area is also equally important. The coarseness and grinding efficiency will improve as the proportion of open area does.

Table 2. Effect of screen hole diameter of hammer mill on the particle size distribution of maize and wheat

Cereal	**Screen hole, mm**	**< 0.2/0.25 mm**	**0.2 - 0.5/0.6 mm**	**0.5 - 1.0/1.1 mm**	**1.0 - 2.0/2.3 mm**	**> 2.0 mm**	**References**
Maize	1	33	49	18	0	0	(Amerah et al., 2008a)
Maize	7	19	28	27	20	6	
Maize	2	9	39	41	11	0	(Naderinejad et al., 2016)
Maize	5	8	28	29	28	7	
Maize	8	5	24	23	31	16	
Maize	3.18	10	16	34	39	1	(Parsons et al., 2006)
Maize	4.76	4	23	24	46	3	
Maize	6.35	3	18	22	50	7	
Maize	7.94	4	18	24	40	14	

Wheat	1	42	38	20	0	0	(Amerah et al., 2008a)
Wheat	7	8	11	23	48	10	
Wheat	3	14	20	32	33	1	(Svihus, Kløvstad, et al., 2004)
Wheat	6	9	12	21	40	17	
Wheat	3	16	18	26	36	4	(Amerah et al., 2008b)
Wheat	7	10	9	18	50	13	

1.1.4 Effect of microstructure in gizzard development and functionalities

Poultry is a monogastric animal with a unique digestive tract compared to other domestic animals, such as crop, muscular gizzard, bifurcated caeca, and cloaca. The growth, production, and health of poultry depend on proper nutrition. Depending on the age and level of production of the bird, different energy requirements are required. In order for the birds to maintain their health and be able to reach their full potential for productivity, it is crucial to provide appropriate nutrients. Poultry requires high-quality feed, where nutrients are readily available to the digestive enzymes. Besides feed composition, as the most important factor that determines the efficiency of feed utilization by poultry, feed structure and feed form are also important for optimal nutrient utilization.

The digestive tract adjusts quickly to changes in diet structure; this is a well-known phenomenon. To increase feed production efficiency, the appropriate level of grinding must be discovered before feeding the birds, one that maximizes all nutritional and technical benefits while minimizing negative health impacts. Broiler chickens are frequently given ad libitum finely ground and pelleted feeds. Finely ground feed ingredients were traditionally assumed to boost nutrient utilization and growth efficiency by increasing the surface area available for digestive enzymes and improving pellet quality (Behnke, 2001). On the other hand, it is becoming more widely acknowledged that broilers may need a certain amount of physical structure in their diet to improve bird performance (Hetland et al., 2002; Svihus, Juvik, et al., 2004), improving the function of the gizzard, which is known as the "pacemaker" of gut motility (Duke et al., 1977; Ferket & Gernat, 2006), and to develop their digestive physiology. Due to their effects on the growth and functionality of the gizzard, dietary structural components including whole grains and coarse particles have received a lot of attention recently when used in poultry diets (Svihus et al., 2002). Such techniques' advantageous benefits may also include their favorable impact on the gut morphology and microbial profile (Engberg et al., 2004). The structure of feed, microstructure and macrostructure, are defined in the introduction chapter. Here, in this section, the microstructure of the diet and its effect on gizzard development is more focused.

The gizzard is a dynamic organ that changes in size in response to stimulation and reacts quickly to dietary changes, especially changes in the structure of the food, by increasing organ mass, muscle mass, or both (Abdollahi et al., 2019). To develop appropriately, the gizzard only needs to be stimulated by coarse particles, and failure to do so results in a tiny gizzard that is incapable of grinding large particles or performing other critical duties. In a poorly developed gizzard, the retention time is too short, decreasing the digestive and sterilizing functions of this compartment. Thus, when the whole grains of wheat were added to the mixture for feeding one-day broilers, Biggs and Parsons (2009) discovered significant gizzard enlargement at 7 days of age. According to data from research, enlargement can be up to 100% and is a logical result of the need for ground feed (Svihus, 2011). Observed investigations have indicated that the capacity of the gizzard increased as soon as the structural components of the diet got underway. In comparison to the growth in gizzard size, this rise is more apparent (Hetland et al., 2003). When the diets were in mash form, Nir and Ptichi (2001) found a positive correlation between the relative gizzard weight and feed particle size. Digesta was held in the gizzard for longer in birds fed mash diets with coarsely ground particles, concomitant with higher gizzard development (Engberg et al., 2002; Hetland & Svihus, 2001). Day-old chicks fed medium and coarse maize particles had gizzard weights that were 26 and 41% heavier than those fed fine particles, according to Nir, Hillel, et al. (1994). Similar findings were made by Charbeneau and Roberson (2004) and Jacobs et al. (2010) about an increase in gizzard weight when dietary maize particle size increased. The impact of soybean meal and maize particle sizes on gizzard weight was compared by Pacheco et al. (2013). They discovered that maize particle size had a stronger impact on gizzard size than soybean meal particle size. At 49 days of age, Xu, Stark, Ferket, Williams, Nusairat, et al. (2015) discovered that the addition of 500 g/kg of coarse maize lowered the relative proventriculus weight and raised the relative gizzard weight. It was proposed that the association between the weight of the gizzard and proventriculus might be explained by the possibility that broilers may have modified their enzymatic and mechanical digestion processes in response to the physical structure of feed. A logical result of the increased mechanical grinding activity is an increase in gizzard weight caused by larger grains of maize (Dahlke et al., 2003; Parsons et al., 2006). According to Naderinejad et al. (2016), medium and coarse grinding of maize enhanced the gizzard weight compared to fine grinding, regardless of feed form.

It has been consistently demonstrated that the pH of the gizzard content drops by a magnitude of 0.2 to 1.2 units when structural components, such as whole or coarsely ground grains, or fiber materials, such as hulls or wood shavings, are introduced (Sacranie et al., 2012; Senkoylu et al., 2009; Svihus et al., 2013). Lower pH levels were seen in the gizzard of broilers fed coarsely structured diets as opposed to feeds that had been processed more finely (Engberg et al., 2002; Jiménez-Moreno et al., 2013; Nir, Hillel, et al., 1994). According to Jiménez-Moreno et al. (2013); Jiménez-Moreno et al. (2009) and González-Alvarado et al. (2008), adding structural ingredients such as oat hulls and sugar beet pulp to mash diets causes the gizzard's pH to decrease. Sacranie et al. (2012) reported the same outcome for a mix of oat and barley hulls. The increased gizzard volume, which results in a longer retention period and more hydrochloric acid secretion, is the logical explanation for this. High feed intake is likely to result in a raised gizzard pH since feed often has a pH that is close to neutral unless gastric juice secretion is able to increase in conjunction with intake (Svihus, 2014). The increase in gizzard size when the diet contains structural components in the form of coarse fibers or cereals improves digestive performance both through an increased retention time, a lower pH, and better grinding, as detailed in great detail by Svihus (2011). It has been demonstrated that this enhances nutrient utilization, possibly in conjunction with better feed flow synchronization. The graphical representation in figure 3 shows the mechanism of how fibers, coarse particles, or whole-grain in the poultry diets modulate gut function and performance (Kheravii et al., 2018).

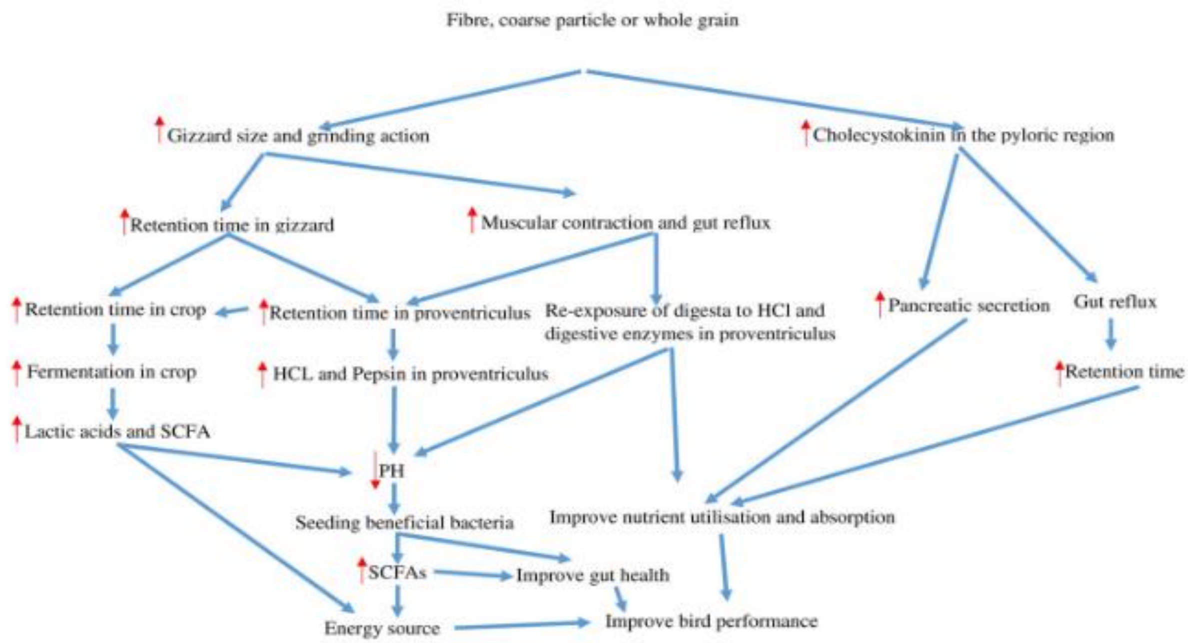

Figure 3. Possible explanations for increased nutrient absorption, performance, and intestinal health via dietary modulation of structural elements (Kheravii et al., 2018)

The minimum level of coarse particles that must be consumed to increase gizzard mass is unknown. In order to promote gizzard development, Svihus (2011), in a review paper on gizzard functionality, advised that at least 20% of the feed particles in the diet be larger than 1.5–2.0 mm in size. This suggests that, from a nutritional standpoint, poultry cereals can be ground coarser than is currently the case (Svihus, 2006). It has been shown that a diet comprising up to 40% whole grains can be as thoroughly digested as a diet containing ground cereals (Svihus, Juvik, et al., 2004). Also, by substituting whole wheat or barley for ground wheat or barley, starch digestibility was improved ($P < 0.05$) (Hetland et al., 2002).

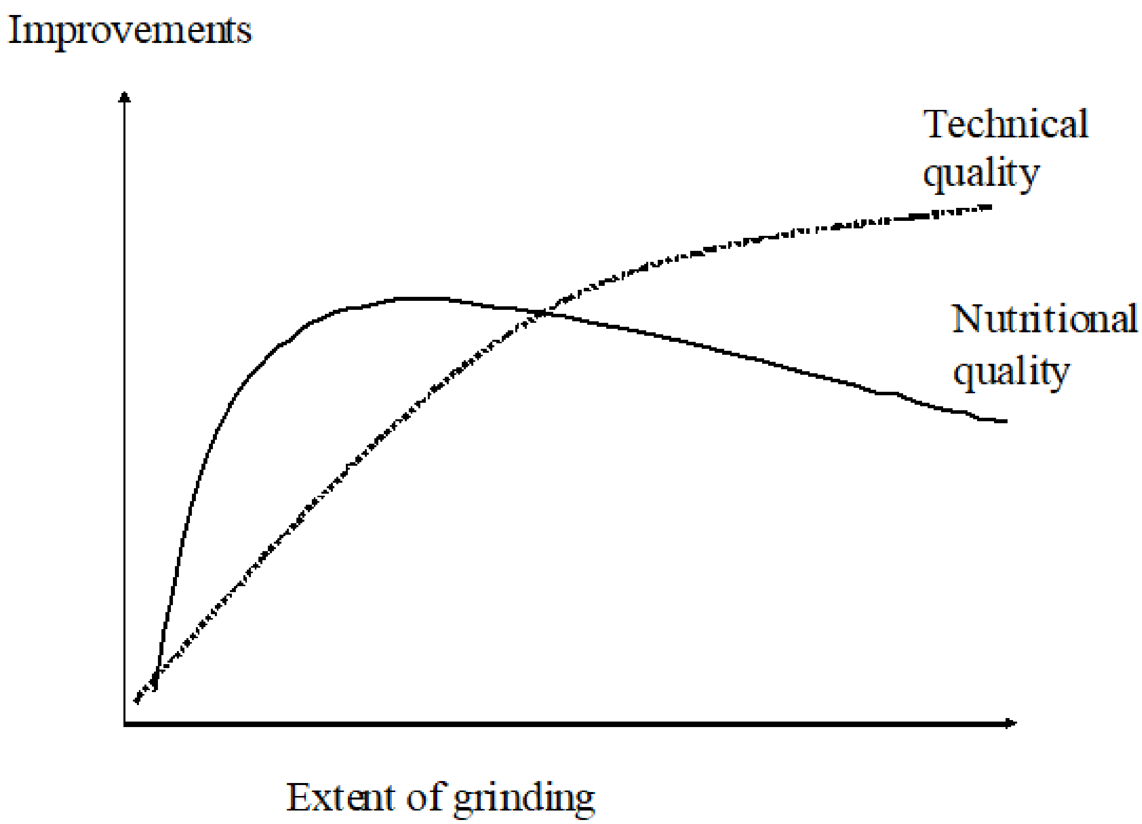

Figure 4. Relationship between improvement in the technical and nutritional quality of feed with increasing extent of grinding.

Because many animal species appear to have an optimal level of grinding, and the rule for technical excellence appears to be the finer, the better, the advantages of grinding can be represented as shown in figure 4. Grinding increases the nutritional value up to a point, beyond which it diminishes. Technical quality, on the other hand, will likely improve consistently as grinding becomes finer and finer. As a result, there appears to be a risk that diets are too finely ground to be nutritionally beneficial.

1.1.5 Mixing

There are two primary approaches for proportioning: batch and continuous. Ingredients are individually weighed into batches in a cyclical or batching system, whereas ingredients are added concurrently and continuously in a continuous system. Thus, proper mixing of the ingredients is essential to generate a homogeneous mixture of proportionate ingredients (Abdollahi et al., 2013b). Mixing is a fundamental process in feed manufacturing, and it is the only operation required to define a feed mill. When ingredients are combined to be fed as a full meal, they must be mixed, and nutrient homogeneity in a complete diet is required to maximize nutrient use, as one might expect. Animals should be fed a balanced diet that includes the right nutrients and feeds additives in the right amounts to maximize growth, production, and health (Behnke & Beyer, 2002). Because chicks only ingest a few grams of feed per day, all critical nutrients must be present at the right levels in a very little meal. Despite a thorough assessment of the literature, little quantitative data on the impact of poor feed mixing on subsequent animal performance was observed (Behnke & Beyer, 2002).

1.1.6 Steam conditioning and pelleting

The procedure of steam-conditioning mash before pelleting is an important stage in the pelleting process (Skoch et al., 1981). A proper balance of heat and moisture must be achieved to optimize the conditioning process. Steam-conditioning at 65 and 78 °C enhanced pellet production rate by 250 and 275 percent, respectively, above dry-conditioning at 21 °C, according to Skoch et al. (1981). Steam-conditioning also increased pellet quality, as determined by the pellet durability index (PDI) (90.6 and 93.8 percent in steam-conditioning at 65 and 78 °C, respectively, compared to 69.5 percent in dry-conditioning) (Abdollahi et al., 2013b).

Pelleting has grown in importance in the feed industry since its inception in the 1930s (Behnke, 1996). Pelleting is the most common method of producing feed for broilers and piglets, nowadays. The pelleting process can be characterized as the mechanical agglomeration of small particles into larger particles in the presence of moisture, heat, and pressure. The mash feed is passed from the mash bin to the feeder and conditioner in this step. Conditioned mash flows into the pelleting chamber after steam injection into the feed inside the conditioner. Pellets are made by forcing heated mash through a metal die as shown in figure 5 and then cooling it. Depending on the product being processed, cooled and dry pellets exit the cooler and travel around or through the crumbler (Abdollahi et al., 2013b).

Figure 5. Pellet mill die chamber

Retrieved from: https://www.allaboutfeed.net/animal-feed/feed-processing/pelletings-role-in-producing-effective-feeds/

Pelleting helps animals perform better and improve feed conversion. The motivation for pelleting according to Behnke (1994) are:

1. Feed wastage and selective feeding were reduced.
2. Less segregation of ingredients
3. Reduction in the amount of time and energy spent on prehension
4. Pathogenic organisms are destroyed
5. Thermal modification (starch gelatinization and protein denaturation improved)
6. Increased palatability

Historically, research has mostly focused on the advantages of feeding pellets vs whole grain or mash diet. The practice of post-pelleting mixing coarse particles with pellets is emerging nowadays.

As previously mentioned, diet particle size can be optimized during the grinding process; however, if poultry feed manufacturing includes downstream processing processes like pelleting, extrusion, or expansion, particle size can be drastically altered. Pelleting binds particles in larger agglomerates while also reducing particle size (Svihus, Kløvstad, et al., 2004). At the stage where feed material is compressed between the rollers and the die to force the material into the holes, the pellet press will serve as a secondary grinder after hammer or roller mill grindings. The particle distribution of the feed mash and the pellet press parameters determine the grinding action. Larger die holes will lessen the pellet press's grinding effect. Wet sieving comparisons revealed that pelleting raised the fraction of tiny particles <0.075 mm in feed samples and decreased the relative proportion of coarse particles >2 mm as shown in figure 6 (Abdollahi et al., 2011). Pelleting is not a suitable approach to achieving the ideal coarser particle in the feed. Instead, the post-pelleting technique of mixing whole cereals or coarsely ground structures might help to preserve the microstructure, which in turn improves feed intake and gizzard functionality.

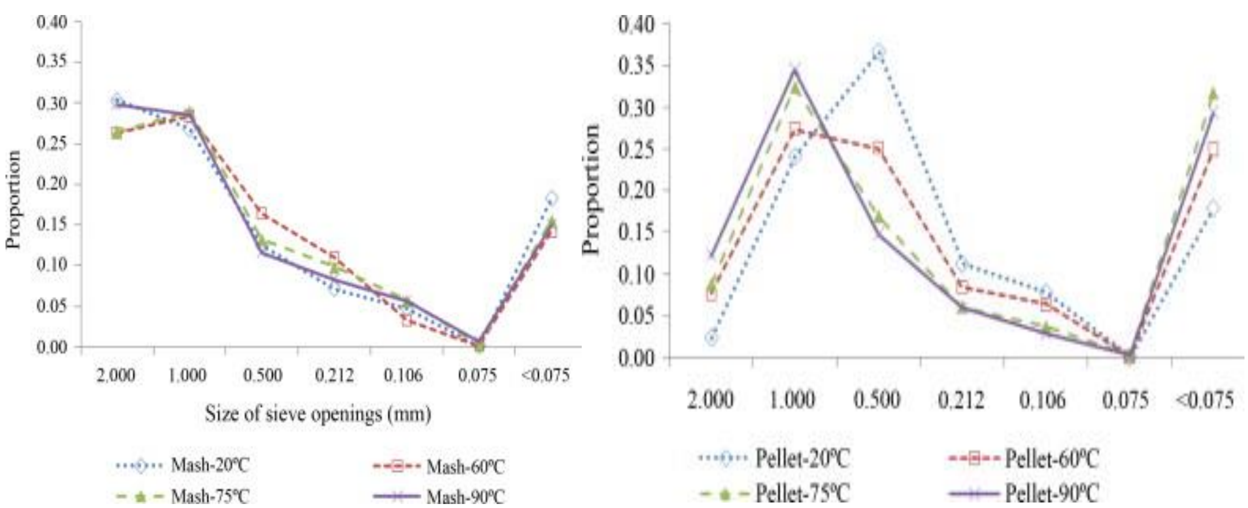

Figure 6. Particle size distribution of mash and pelleted diets at different temperatures (Abdollahi et al., 2011).

1.1.7 Effect of feed particle size on pellet quality

The ability of a pellet to withstand abrasion and fragmentation during handling without breaking up and to reach feeders without producing a significant amount of particles is known as pellet quality (Amerah, Ravindran, et al., 2007a). One of the key factors used to assess the quality of pellets is the pellet durability index (PDI), which shows the proportion of pellets that stay intact after being subjected to mechanical stresses. Poor-quality pellets disintegrate during storage, transit, and dispatch from the feed mill to the farms (Lowe, 2005; Mina-Boac et al., 2006), resulting in a feed consisting of a few pellets and fines. Pellets are subjected to friction, impact, and pressure throughout these processes. Pellet quality is influenced by several variables, including feed formulation, feed particle size, conditioning time and temperature, feed moisture content, pellet die compression rate, and the gap between the pellet press roll and die (Čolović et al., 2010). Additionally, there might be interactions among these variables, leading to outcomes that diverge from what would be predicted when individual parameters are taken into account.

Published researches on the effect of feed particle size on pellet durability are conflicting. The prevailing assumption is that the durability of pellets and particle size have an antagonistic relationship (Angulo et al., 1996) because smaller particles have more contact points with one another due to a higher surface area-to-unit volume ratio (Behnke, 2001). According to Thomas et al. (1998), coarser particles produce weak spots that make pellet breakage easier and reduce pellet quality. Svihus, Kløvstad, et al. (2004) found that a wheat-based diet made from coarse particles was slightly less durable than diets made from fine particles due to more weak spots in pellets made from coarsely ground wheat In contrast, Reece et al. (1986) showed that increasing the coarser particles in the diet markedly increased the pellet durability. No differences were found between the pellet durability index of pellets made from diets based on medium- (3 mm screen size) and coarsely ground cereals (7 mm screen size) by Amerah, Ravindran, et al. (2007b). Stevens (1987) observed that the durability of pellets made from various particle sizes of maize was identical, however, the durability of pellets made from wheat was lower when coarser particles were used. In contrast to coarse oat hulls, Zimonja et al. (2008) found that adding finely ground oat hulls increased pellet durability. Although there is no conclusive evidence to back this assertion, it is thought that coarsely ground particles degrade the quality of the pellets (Amerah, Ravindran, et al., 2007b).

1.1.8 Effect of macrostructure (pelleted diets) on feed intake and performance

The feeding behavior and the unique digestive system are the important factors that should be taken into consideration while optimizing feed for poultry. Taste and smell are not the primary stimuli for feed intake in farm animals, in contrast to many other farm animals (Neves et al., 2014). Instead, when feeding, the bird places a strong emphasis on visual cues. As a result, feeding behavior may be greatly influenced by form. The bird also probes the food with its beak in a tactile manner (Ferket & Gernat, 2006). Feed intake is accomplished by grabbing the food particles with the beak and swallowing them instantly without much chewing or saliva mixing. All of these traits of chickens suggest that the optimum feed should contain particles of a specific size and shape to encourage and maximize feed intake. So, the primary goal of the pelleting process is to shape the feed ingredients into big particles in order to improve handling qualities and ensure that the poultry consumes the feed efficiently and uniformly. Reduced feed ingredient separation, feed wastage, starch gelatinization, and increased palatability are further major advantages of pelleted feed (Behnke & Beyer, 2002).

It has long been demonstrated that feeding broilers pelleted diets, regardless of the kind of cereals or the age of the birds, increases growth rate and feed efficiency (Abdollahi et al., 2011; Engberg et al., 2002; Latshaw & Moritz, 2009). Numerous variables, such as greater nutritional density, less selective feeding, feed waste, and less time and energy expended on feed consumption, have been cited as the causes of these improvements (Jensen, 2000). The enhanced performance might be, to a large measure, attributed to the stimulatory effect of pellet feeding on feed intake because the favorable effects of pelleting on broiler growth match the effect on feed consumption (Abdollahi et al., 2014). The higher feed consumption in pellet-fed birds may be partly attributed to the rise in bulk density of pelleted diets, which makes easy prehension possible. When compared to broilers fed mash feed, broilers fed pelleted feed has better performance such as feed intake (FI), body weight gain (BWG), feed conversion ratio (FCR), etc. (Abdollahi et al., 2013a; Bergeron et al., 2018; Chewning et al., 2012; Corzo et al., 2011; Nir et al., 1995). Due to the pelleting of broiler diets, increased FI has been reported to vary from 2.8% (Serrano et al., 2012) to 64% (Amerah, Ravindran, et al., 2007b).

Pelleting improves feed efficiency in part because less feed energy is required for maintenance. Therefore, pelleting may result in increased productive energy (Nir, Twina, et al., 1994). An estimate of the MJ per unit of feed actually used for fat and protein accretion is known as productive energy (Reddy et al., 1962). According to Reddy et al. (1961), chickens fed pellets consumed their food for about 4% of the time as opposed to 15% of the time for birds fed mash. According to a study by Latshaw and Moritz (2009), the feed form had an impact on how much energy from each unit of feed was used for production and heat increment. Compared to broilers given mash, those fed pellets exhibited lower heat increment and used more of the feed energy for productive purposes. Therefore, feed intake will increase when broiler diets are changed from mash to pellets, which improves feed efficiency.

The impact of pellet quality on bird performance is widely acknowledged. Male broilers fed pelleted diets containing 45 percent fines had a 150 g lower 49-d body weight (BW) than broilers fed pelleted diets without fines, according to Proudfoot and Sefton (1978). In a comparison of two different pellet textures, namely soft (1662 g of pellet breaking force) and hard (1856 g of pellet breaking force), Parsons et al. (2006) found that broilers fed hard pellets had improved weight gain and feed efficiency compared to those fed soft pellets. Also, broilers' behavior changed when they were given high-quality pelleted feed. Broilers raised their resting frequency and decreased their eating frequency when given high-quality pellets (Skinner-Noble et al., 2005). Lilly et al. (2011) found that high and medium-quality pellets led to enhanced FI and BW gain when compared to low-quality pellets and ground pellets when comparing pellets of high, medium, and low quality to one other. In addition, a pellet in any form outperformed ground pellets in terms of geed per gain.

Numerous studies have shown that giving diets comprising cereals ground to various sizes resulted in equivalent feed intake whether the diets were pelleted or crumbled as shown in table 3. Experiments with pelleted diets comprising cereals ground to various sizes reveal that coarse grinding produces comparable weight gain and feed utilization as fine grinding (Hamilton & Kennie, 1997; Nir et al., 1995).

Table 3. Influence of particle size on the feed intake (FI, g/bird) of broilers fed pelleted or crumbled diets

Grain type	Age	Screen size (mm)	Particle size GMD (µm)	Feed Intake (FI)	References
Maize	1-21	2	253	1379	(Naderinejad et al., 2016)
		5	275	1373	
		8	299	1368	
Maize	1-21	1	NR	1191	(Amerah et al., 2008a)

		7	NR	1173	
Wheat	1-21	3	NR	1271	(Amerah, Ravindran, et al., 2007b)
		7	NR	1253	
Wheat	1-21	1	NR	1357	(Amerah et al., 2008a)
		7	NR	1262	

NR: Not reported; GMD: Geometric mean diameter.

1.1.9 Effect of feed form in feeding behavior and feed intake

When assessing the impact of particle size on poultry performance, uniformity of particle size bears an equal weight to feed particle size (Amerah, Ravindran, et al., 2007b). Both may influence the performance of poultry (Axe, 1995). Birds have the ability to discern changes in feed particle size using mechanoreceptors found in the beak, which is crucial for their performance (Gentle, 1979). All studies may agree on one point, which is demonstrated by the fact that because of their digestive systems, chickens are known to prefer larger feed particles (Schiffman, 1968). Particle size preference may be influenced by the fact that birds have difficulties eating food that is larger or smaller than the dimensions of their beaks. As mentioned earlier, it appears that birds' predilection for bigger particles increases with age (Moran, 1982).

Particle size and diet composition may have an impact on the patterns of diurnal feed consumption in birds, according to Savory (1980). According to Schiffman (1969), birds prefer pecking at textured feed over non-textured feed. Fujita (1973) discovered that birds given pellets as opposed to mash or crumbles had a more noticeable feeding behavior. Despite spending less time at the feed trough when fed pellets, birds' daily meal intake was the same regardless of particle size. Although none of the feed forms significantly influenced daily feed intake, there was clear proof that feed granulation reduced the amount of time spent eating (Fujita, 1973). A significant amount of feed was eaten in a noticeably shorter period of pecking by the birds getting pellets as opposed to mash, which was seen to be nearly continually present at the feed trough during daylight hours. There was a clear tendency for the feeding activity to increase at first after delivering fresh mash when the birds got the standard type of commercial mash ration (Fujita, 1973). According to Jensen et al. (1962), there was no change in the number of diets consumed each day, even though the average time spent eating mash was longer than that spent eating pellets. Allen and Perry (1977) observed that the percentage of particles larger than 2.0 mm steadily decreased over time, whereas the concentration of particles smaller than 1.0 mm rose. Similar outcomes were obtained by Portella et al. (1988) when working with laying hens, however, these researchers noted that small particles gradually vanish in the absence of large particles. Portella et al. (1988) while working with broilers found that, at all ages, particles larger than 1.18 mm had disappeared and the elimination of particles between 1.18 mm and 2.36 mm was found to be most prominent at 8 and 16 days. The rate of disappearance was highest for particles larger than 2.36 mm as broilers aged. Also, Portella et al. (1988) in the same experiment found that changing the particle size abruptly from a crumbled to a pelleted diet did not make a significant difference in the feed intake of broiler chickens. When it comes to pelleted diets, the diameter and length of the pellets are also important factors responsible for feed intake. Several experiments with wheat- or maize-based diets have demonstrated that broiler chickens performed just as well when the pellet diameter was 4.4 to 4.76 mm as when the diameter was 3mm (Abdollahi et al., 2012; Singh & Ravindran, 2014).

The degree of cereals grinding is critical when assessing the impact of feed form on the feed intake of broilers. According to reports, weight gain and feed per gain improved when fed coarse particles compared to the medium particles in the mash diets, (Amerah, Ravindran, et al., 2007b; Nir et al., 1995). Amerah, Ravindran, et al. (2007b) observed that pelleting significantly increased the feed intake in medium-ground wheat diets compared to coarse-ground wheat diets (64 vs. 43 percent), with matching weight gain responses of 84 and 53%, respectively. The importance of physical quality on bird performance is well recognized in the broiler industry. The feed intake and growth responses in broilers may also be influenced by macro-structural features of pellets, such as physical quality (% of undamaged pellets, durability, and hardness), size (length and diameter), and others (Corzo et al., 2011; Cutlip et al., 2008; Skinner-Noble et al., 2005). Based on the discussion above, it is logical to conclude that any pelleting approach that may enhance pellet physical quality, decrease the proportion of fines, and more critically boost feed intake will likely lead to better nutrient and energy intakes, which will in turn improve growth responses.

Whole grain feeding is an emerging practice in poultry nutrition around the world. The main reasons to feed whole grains to broilers are to reduce feed handling and processing costs and to enhance the growth and functionality of the foregut. One of two methods, namely pre-pelleting or post-pelleting, can be used to include whole grains, most frequently whole wheat, into the pelleted diet. In the pre-pelleting technique, whole grain is added to a pellet in place of some ground grain, but in the post-pelleting technique, whole grain is combined with the pelleted concentrate after other feed ingredients have been mixed and pelleted (referred to as pelleted concentrate) (Abdollahi et al., 2018). Mixing of coarse structures in the diets post-pelleting and whole-grain feeding (Svihus et al., 2010) are thought to have beneficial effects on feed consumption and digestive tract development and functionality. So, post-pelleting mixing of coarsely ground cereals with pellets can also be a new practice in broiler feed production.

Published literature indicates that post-pelleting inclusion of whole wheat (WW) either had no effect or decreased feed intake. The age of the birds at the introduction of whole wheat is critical in terms of responses to this feeding strategy. Ravindran et al. (2006) reported significant decreases in feed intake and weight gain of broilers on day 7 due to the introduction of whole wheat post-pelleting from day 1. They also observed that the chicks had difficulty swallowing whole wheat during the first few days of life. When post-pelleting whole wheat was added from day one, Wu and Ravindran (2004) and Wu et al. (2004) similarly noted a similar decline in feed intake. The compacted whole wheat in avian crops and gizzards may be one factor in the feed intake depression caused by post-pelleting whole wheat inclusion (Abdollahi et al., 2016). With a reduced feed passage rate and compromised feed intake, the whole wheat with a greater grinding demand would be kept longer in the upper gut. As mentioned earlier, the age of the birds and the rate of WW inclusion have a significant impact on the degree of feed intake. There was no noticeable feed intake reduction when WW was added at later ages (Hetland et al., 2003). The effect of mixing the coarse cereals with the pelleted diets as this has not been focused much on the poultry feeding regime. The current experiment with coarser grinding and post-pelleting inclusion of coarser microstructure instead of whole cereals was started after taking into account the lower feed intake and the unsurprising broiler performance on post-pelleting whole wheat inclusion as a reference.

2. Materials and methods

This thesis is divided into two parts, the grinding section and the broilers experiment section.

2.1 Grinding experiment

A test grinding was carried out before formulating and processing the broilers' diets. Four major kinds of cereal (maize, wheat, barley, and oats) were purchased and test grinding was run in the Center for Feed Technology (Fôrtek), Ås, Norway. Representative samples of whole cereals were taken before grindings for the physical measurements (hardness, length, width, and particle size distribution). All four kinds of cereal were ground at 18 amperes in a hammer mill (HM 21.115, M¨unch-Edelstahl, Wuppertal, Germany, licensed by Bliss, USA) to pass through screens of 3, 6, and 8mm that are of current practice. While only maize and wheat were ground using the same hammer mill and the same amperes to pass through the different novel screens. The feeder rate was adjusted to the amperage. In this particular hammer mill, it was not possible to adjust the amperage and the feeder rate was automatically adjusted to get that specific amperage. So, the feeder rate was different in different samples. The hammer mill had 24 hammers with a thickness of 6 mm each and was driven by an 18.5 kW electric motor with a rotational speed of 2870 rpm. The distance between the hammer and the screen was 15 mm, the tip speed was 98 m/s, and the shaft speed was 2870 rpm. The design of the novel screens is confidential due to patent issues. Representative samples were collected after grinding each cereal on each screen. The particle size spectrum of each grade was subsequently characterized by dry sieving. The energy consumption and grinding capacity were noted for each grinding.

2.2 Experimental diets and processing

After conducting the test grindings and subsequent sieving, one normal 3 mm screen and two novel screens (A and B) were selected for the production of the wheat and barley-based experimental diets. Novel Screen A and Novel Screen B were used for the moderately coarse and very coarse grindings, respectively.

The seven wheat and maize-based experimental diets based on a normal 3 mm screen and two novel screens (A and B) were produced at the Center for Feed Technology (Fôrtek), Norwegian University of Life Sciences (NMBU), Ås, Norway. Wheat and maize were ground at 18 amperes in the Muench hammer mill (HM 21.115, M¨unch-Edelstahl, Wuppertal, Germany, licensed by Bliss, USA) fitted with a standard 3mm screen and novel screens with different coarseness. Out of seven diets, three of them were pelleted, and the rest four were heterogeneous diets. Heterogenous diets were a mixture of pellets and coarsely ground cereals. One pelleted diet was made from cereals ground using a normal 3 mm screen (SP) while the rest two pelleted diets were made from very coarsely ground cereals (VCP) and moderately ground cereals (MCP). For the heterogeneous diets, after grinding, the cereals were sieved in the vibrator sieve (JOHS. ØGENDAHLS MASKINFABRIK LEMVIC A/S, Lemvig, Denmark) fitted with a 2 mm sieve to separate fines and coarse particles (called coarse cereal after one sieving). The coarse cereals thus obtained from the first sieving were again sieved using the same vibrator sieve with a 2mm sieve (called coarse cereals after two sievings) to remove more fines. Thus obtained fines from the respective sievings were used to produce pellets. Through a 3mm die with 42 mm thickness, the mash (fines mixed with other premixes) was steamed-conditioned at 75°C for diets SP, VCP, and MCP and 82°C for the remaining diets, at a feeder rate of 800 kg/h for diets SP, VCP, and MCP and 600 kg/h for heterogeneous diets. Following pelleting (Muench, Wuppertal-Germany) and cooling (Miltenz Counter flow cooler, Auckland, New Zealand, 2000 kg/h capacity), pellets were mixed with the coarse fraction of the ground cereals, from one sieving (VCPM1 and MCPM1) and two sievings (VCPM2 and MCPM2) in the twin shaft mixer (TATHAM, United Kingdom). The diets contained 5 g/kg of Titanium dioxide (TiO_2). Corn, wheat, and soybean meal-based diets were formulated to meet the Ross 308 strain recommendations for major nutrients for broiler diets. All the diets had the same compositions, which were based on the g/kg as feed (Table 4).

Table 4. Diets composition.

Ingredients	g/kg as feed
Wheat	433.0
Maize	250.0
Soybean meal	200.0
Maize gluten meal	40.0
Soy oil	36.0
Premix SLK 1.5%	16.0
Limestone meal	09.0
Monocalcium phosphate	06.0
Titanium dioxide (TiO_2)	05.0

Salt	02.0
Sodium bicarbonate	02.0

The percentage of pellets, coarse maize, and coarse wheat in the finished diets were according to the formulation mentioned below and had the following specific characteristics:

Diet SP: Standard Pelleted control diet which was ground on a 3 mm hammer mill screen and pelleted following current standard practice

Diet VCP: Pelleted diet based on very coarsely (VC) ground cereals

Diet VCPM1: Mix of pellet and the coarse cereal fraction from VC grinding after one sieving (60.1 % pellets, 27.8 % coarse wheat, and 12.1 % coarse maize)

Diet VCPM2: Mix of pellet and the coarse cereal fraction from VC grinding after two sievings (64.9 % pellets, 26.0 % coarse wheat, and 9.1 % coarse maize)

Diet MCP: Pelleted diet based on moderately coarsely (MC) ground cereals

Diet MCPM1: Mix of pellet and the coarse cereal fraction from MC grinding after one sieving (63.3 % pellets, 26.5 % coarse wheat, and 10.2 % coarse maize)

Diet MCPM2: Mix of pellet and the coarse cereal fraction from MC grinding after two sievings (70.6 % pellets, 22.2 % coarse wheat, and 7.2 % coarse maize)

Abbreviations: SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

The analyzed nutrient composition of the seven diets used in an experiment is given in Table 5.

Table 5. Analysed chemical composition (%) of the experimental diets

Diets	Dry matter (%)	Ash (%)	Kjeldahl N (%)	Starch (%)	Crude fat (%)
SP	87.6	4.2	3.1	39.9	5.1
VCP	88.4	4.6	3.2	38.6	5.5
VCPM1	88.8	4.1	3.0	39.1	5.1
VCPM2	88.1	4.4	3.0	37.0	5.3
MCP	88.2	4.6	3.2	38.6	5.5
MCPM1	88.0	4.0	3.0	39.7	5.0
MCPM2	88.0	4.1	3.1	39.6	5.1

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

2.3 Chicken experiment

2.3.1 Experimental design

A total of 1.6 tons of each diet were produced and delivered to the poultry house. All diets were identical in terms of ingredients and nutrient composition based on wheat and maize with a difference in the structure of all diets. The seven experimental diets were given ad libitum, and the time without feed was not more than 3 hours during refeeding, sampling, etc. All the feeders were allowed to become empty before refeeding. Until day 10 (31st May 2021), all birds were given a commercial starter diet from Fiskå mølle, *ad libitum*. On the 10th day, the starter diets left in the feeders were removed, and birds in nine pens distributed across the room were given each of the experimental diets (in total 63 pens).

2.3.2 Bird husbandry

A total of 3150, plus extra to correct for mortality to 10 days, day-old Male Ross 308 broiler chicks arrived on 21st May 2021 in the poultry house of NMBU. The day-old chicks were mixed before placing randomly in each of the 63 pens with 50 healthy birds per pen. The main purpose of mixing was to avoid errors due to different parent flocks. The floor had wood shavings as litter. Each pen was equipped with one drinker line and two feeders as shown in figure 7. The average temperature was 33.8 °C on day 1 and was reduced by 1°C every 2 d until 21 to provide comfort throughout the study. Lightening was 23 hours on the first day in all pens and from day 2, eighteen hours of light and six hours of darkness were maintained. Free access to the water, was made available throughout the trial period.

Figure 7. Pen fitted with two feeders and one drinker line

2.3.3 Feeding behaviour trial

2.3.3.1 Feed spillage and excreta collection

On day 20, 1x1 meter paper sheets were placed below the 27 feeders (figure 8) given diets VCP, VCPM1, and VCPM2 in the morning. After 4 hours, 30-80 gram clean excreta were collected from the paper sheets. The reason for selecting these treatments was to analyze the difference in particle size distribution in excreta when fed a pelleted compared to a heterogeneous diet. The samples were then kept in the freezer until analysis. For feed spillage, the papers were checked carefully under each feeder in 27 pens and no loss was found after 4 hours, so samples were not collected.

Figure 8. Paper under each feeder for excreta and feed spillage collection

2.3.3.2 Behaviour observation

On day 20 from 09:30 to 14:00, the feeding behavior of the birds was observed in the pens where diets MCP, MCPM1, and MCPM2 were offered. Birds were observed with minimal disturbance, sitting on the stool next to the pen and the number of the birds eating or standing close and facing toward the feeder was recorded each minute for 4 minutes. Then, the sum of four minutes in each pen was calculated and the percentage of birds eating was calculated by dividing the total number of birds observed in four minutes by the total number of birds per pen on that day. To assess how much time birds spend eating either pelleted or heterogenous diets, these three diets were chosen.

2.3.3.3 Collection of feed samples from the feeder

On day 27 at 9.30 in the morning, the feeders of the 18 pens given diets VCPM1 and MCPM2 were emptied, refilled, and left high. To compare the differences in particle size selection behavior between two extents of grinding (very coarse and moderately coarse) and the extent of sieving (one sieving and two sievings), these two diets were chosen. First, representative samples from one of the feeders per pen were collected and placed in the zip bag as marked and then the feeders were lowered for birds' access. Then after 1, 2, 3, and 4 hours, all the feed in one of the six sections of the feeder was collected, making sure a new section was selected new hour. Each section was marked after collection to avoid repetition as shown in figure 9.

Figure 9. Different sections of the feeder

2.3.4 Dissection and sample collection on day 32

One bird per pen was randomly selected and was given a cranial blow followed by cervical dislocation. The killed weight was taken and the body cavity was opened to collect and record the gizzard with contents, empty gizzard weight (without peri-gizzard fat and contents), and pH. The pH was measured by inserting a probe into the core of the gizzard through the anterior opening. Samples from the duodenum, anterior jejunum (AJ, posterior jejunum (PJ), anterior ileum (AI), and posterior ileum (PI) were collected and stored immediately in the freezer at -20°C. The jejunum and ileum were defined based on Meckel's diverticulum as the reference point. The graphical presentation of the sampling day is shown in figure 10.

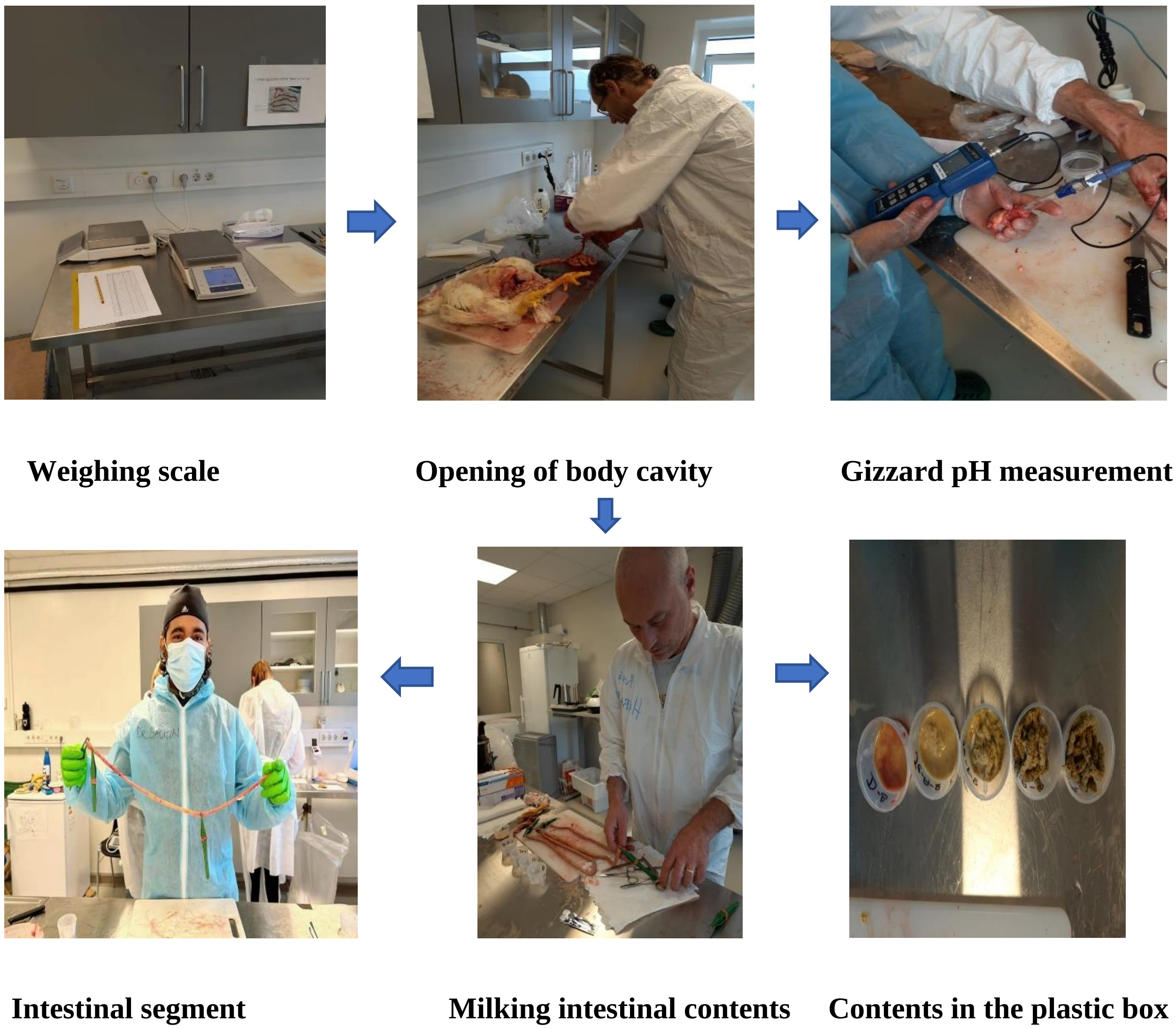

Figure 10. Overview of sample collection day

2.3.5 Measurement of coefficient of variation

On day 34, all the remaining birds were sent to the slaughterhouse and were slaughtered as a batch according to the treatment. The weight of each bird was recorded and the coefficient of variation (CV, %) was calculated based on the weight of all approved slaughtered birds from each treatment. Due to technical errors, the weight of birds fed diet MCPM1 was not recorded in the slaughter so the data is missing in the result. The coefficient of variation was calculated as the standard deviation in a percent of the mean for all the slaughter weights of all the birds in each treatment.

2.4 Chemical analysis

Representative feed samples were processed through 0.5 and 1 mm sieves in a cutting mill (Pulverisette 19, Fritsch Industriestr. 8, 55743 Idar-Oberstein, Germany), while excreta and ileal content were pulverized using a mortar and pestle. The dry matter and ash content of feed and intestinal content was determined after drying at 105°C overnight and ashing at 550°C for 12 hours, respectively. The Kjeldahl technique was used to determine the crude protein. TiO2 content of the feed, excreta, jejunal, and ileal contents were determined as described by Short et al. (1996). Freeze-dried 100 mg of digesta sample (300mg of feed) was weighted and ashed at 550°C and then 10ml sulphuric acid, 7.4 M was added before boiling for 1 hour. And then 20 ml of hydrogen peroxide (30% volume) was added, giving the characteristic orange color, the intensity of which was depending on the titanium concentration of the samples. An UV spectrophotometer was used to measure the absorbance at 410 nm in aliquots of the solutions obtained and of similarly prepared standard solutions. For starch analysis, each tube containing 100 ±5 mg of ground feed, pulverized dried excreta, or freeze-dried intestinal content received 7-8 ml of 80 percent ethanol. The mixture was vortexed for 5-10 seconds and incubated at 80°C for 5 minutes before centrifuging for 10 minutes at 3000 rpm and discarding the supernatant containing mono-, di-, and tiny oligosaccharides. This treatment was carried out twice. Following that, the starch content was measured enzymatically using thermostable-amylase and amyloglucosidase, as described by McCleary et al. (1994).

Apparent nutrient (starch and protein) digestibility was calculated using the following formula:

= 100 - 100*((TiO2 in diet/TiO2 in digesta)*(Nutrient in digesta/Nutrient in diet))

2.5 Physical quality analysis

A detailed procedure that was followed during the physical quality test is explained in the following headings. Pellet durability test, hardness test, and measurement of length and diameter were carried out.

2.5.1 Preparing sample

A sample divider was used to separate one sample bag (ground cereals or experimental diets) into two sections. One clean sieve collector was placed beneath the sample divider first. Then the samples were slowly poured into the sample divider from the top hole (figure 11). Then, using a plastic container, the desired amount of sample based on the tests was taken by scooping small amounts (about 10-15 g) from various locations under the collector.

Figure 11. Sample divider for taking representative samples

2.5.2 Pellet Durability Index (PDI) using Holmen Pellet Tester

To remove fines and dust, 120 to 150 g of samples were gently sieved on a 2mm sieve for 1 minute at an amplitude of 1.5mm. Then the durability was measured using a Holmen Pellet Tester (New Holmen NHP200, TekPro Ltd., Willow Park, Norfolk, UK) (figure 12), where dust-free feed samples weighing approximately 100 ± 5 grams were conveyed pneumatically in a closed circuit for around 80 seconds. Inside the Holmen tester, pellets were blown out via a pre-installed 2.5 mm sieve. The pellet durability index (PDI) was displayed on the tester's screen following automatic testing.

Figure 12. Holmen tester

2.5.3 Length, hardness, and diameter measurement

A random scoop (about 30 pellets and whole cereals) was taken from the sample bag and placed on a flat surface in ascending length order (measuring with the caliper). Then, the middle fifteen pellets and cereals, plus the shortest and the longest pellets and cereals, out of 30 were chosen and their length and diameter were measured with a digital caliper (figure 13), taking care not to apply extra force to the pellets before the hardness test.

The Kahl-Hardness tester (Amandus Kahl Gmbh Co.) fitted with a 2.5 mm spring was used to measure the hardness of the pellets and whole cereals (figure 14). To begin, the indicator was set to "0" by slightly reversing the piston then positioning the testing pellets between the anvil and the piston and tightening the fixing screw until there was a slightly larger space between them than the diameter of a specific sample. The pressure screw was tightened until the pellet or grains were crushed and, the pressure used to break the pellets or cereals (in kg) was recorded. Then the pressure screw was removed and the previous steps were repeated for the remaining pellets or cereals.

Figure 13. Vernier caliper used for length and diameter measurement

Figure 14. Kahl-hardness tester for pellets hardness measurement

2.6 Determination of particle size distribution

Particle size distribution was carried out using dry sieving, wet sieving, and the laser diffraction method (Mastersizer). Dry sieving was performed in all the samples collected from test grindings in addition to the experimental diets (mash and finished diets) and feed that was collected from the feeders every hour for hours. Results of mash diets are not shown in this report. Wet sieving was done on the excreta samples, gizzard contents, three mash diets (SP, VCP, and MCP), and seven experimental diets. In order to determine the particle size of the contents of the duodenum, jejunum, and ileum, the Mastersizer 2000 instrument was used. A detailed procedure of each technique is mentioned in section 2.6.1.

A. Samples from test grindings and experimental diets

Dry sieving was performed in all the samples that were ground during the test grindings. 100 grams of samples were taken with the help of a sample divider and using 3.55 mm, 2.8 mm, 2 mm, 1.6 mm, 1 mm, 0.5 mm, 0.1 mm sieves, and a base-collector, the test was performed. The detailed procedure is described in the 2.6.1.1 section below.

B. Gizzard contents and litter separation

A wet sieving procedure was carried out to determine the particle size of the gizzard contents in diets SP, MCP, and MCPM1. The reason for selecting these three diets was to compare the litter consumption behavior between finely ground and coarsely ground diets. Each treatment's gizzard contents were pooled into two separate sub-samples at random. Because of the limited sample quantity, four samples randomly from each of the nine replicates (per diet) were combined to create one sub-sample, and the remaining five samples were pooled to create a second sub-sample. Then, the wet sieving procedure (after soaking in 500 ml of water for 1 hour) using sieves of 2.8mm, 2mm, 1.6mm, 1mm, and 0.5mm diameter was performed as described below. After oven drying and weighing for gizzard contents, the litter separation from each sieve (2.8mm, 2mm, 1.6mm, and 1 mm) was performed. The dried samples from each sieve were spread on the flat and clean surface and then using the pinset, litters were picked/separated from the dried gizzard contents and weighed separately. The percentage of litter in gizzard contents was analyzed using the formula given below. No visible litter was seen in the 0.5mm sieve so was not accounted for.

% of litter in gizzard content = (litter wt. after drying/ dried sample weight)*100%

C. Feed collected from the feeder

Dry sieving was performed to determine the particle size of the feed collected from the feeder every hour for 4 hours. Feed was collected from both feeders within a pen and then the samples were pooled to make one large sample per hour per pen. Then samples from only four pens per treatment were randomly chosen for the dry sieving analysis. The sieves of sizes 2.8mm, 2mm, 1.6mm, 1mm, and 0.5mm were used to perform dry sieving of the samples collected and the amount of samples used for analysis was the total feed collected from each pen.

D. Intestinal contents

The intestinal samples were thawed at room temperature after taking out from the freezer. Only pelleted diets (SP, VCP, and MCP) were used to determine the particle size of the intestinal contents. The reason for selecting only pelleted diets was to avoid non-uniformity in the results due to heterogeneous structure. However, for the ileal particle size analysis, all seven diets were used.

E. Excreta collected

A wet sieving procedure was done to determine the particle size of the excreta collected from the pens fed with diets VCP and VCPM2. The excreta samples were first cleaned by removing the litter or other foreign materials attached to them. Then four pens per treatment were chosen randomly for the particle size distribution analysis. The samples were thawed and soaked in 500 ml water for 1 hour and wet sieved. The percent dry matter weight of the sample retained on each sieve size was calculated. The sieving process was carried out using only 2.8mm, 2mm, 1.6mm, 1mm, and 0.5mm sieves.

2.6.1 Measurement of particle size distribution

2.6.1.1 Dry sieving

a) **Preparing sieves**: Sieves of sizes 3.5mm, 2.8mm, 2mm, 1.6mm, 1mm, 0.5mm, 0.2mm, 0.1mm, and a bottom collector were used. In the case of the samples from feeding trials, some sets of sieves mentioned above were removed as described above in section 2.6 (**C**). All the sieves were cleaned with a brush and an empty weight (initial weight) was recorded. These sieves were stacked in descending order according to sieves' diameter from top to bottom.

b) Approx. 100 grams (depending on the quantity of the samples) of the sample was taken from the sample bag using a sample divider as described earlier and poured into the top sieve. The stacks of the sieves were closed with the top lid and clamps were tightened as shown in figure 15. Then, the sample was shaken for 1 minute at an amplitude of 1.2 mm/g in an analytical sieve shaker (AS 200 control, Retsch, Haan, Germany). The sieves including the bottom collector were thereafter weighed (final weight). The percentage of samples that remained on each sieve was calculated by dividing the weight of the sample (final weight - initial weight) after sieving by the total weight of the sample poured into the sieve set. Finally, all sieves and collectors were cleaned with brushes and re-weighed for the next sample.

Figure 15. Retsch analytical sieve shaker with sieves and lid for dry sieving

2.6.1.2 Wet sieving

a) **Preparing sample**: The amount of sample, water to be used, and the time for soaking was different for different types of the sample as mentioned above in section 2.6. Generally, 100 grams (depending on the quantity of the samples) of the representative sample (mash and finished diets) was taken using a sample divider and soaked in a beaker containing 500 mL of water for 2 hours at room temperature. Stirring was done from time to time after 1 hour of soaking to ensure the complete dissolving of the pellets. In the meantime, the sample for dry matter analysis was prepared. 20 to 30 grams (depending on the quantity of the samples) of the sample were taken from the sample bag and ground with the help of a mortar and pestle as shown in figure 16. Two small trays were weighed and about 20 grams of sample were taken into them and placed in the oven with the sieves. All the measurements were recorded in a single excel sheet made for both wet sieving and dry matter %.

Figure 16. Beaker with dissolving pellets and mortar and pestle with ground sample for dry matter analysis

b) **Preparing sieves**: The same sieves set used in dry sieving were used for wet sieving also except for the bottom collector. The bottom collector connected with the water outlet was used in this process. The rubber bands were put around the lower edge of each sieve to prevent water leakage during sieving time. And all the sieves were stacked in the analytical sieve shaker in descending order from top to bottom.

c) **Wet sieving**: After 2h of soaking, the fully dissolved samples were poured into the sieves from the top. The top of the sieves was covered with a lid designed for the wet sieving process as shown in figure 17. The water valve was turned on and the position of the valve was marked to ensure the same water treatment for all diets. Then the sieve shaker was set at 1.2 mm/g for 3 minutes. Thereafter, the outlet pipe was lifted upwards until the bottom two sieves (0.1 and 0.2 mm) were filled with water. This can be known from either the water line in the outlet pipe or the leakage from the sieves gaps in these two sieves. When those two sieves were full of water, the water valve was turned off and kept holding the outlet upwards, and set the sieve shaker in 1 min. The Sieve shaker was turned on for 1 minute without water. Then, the water outlet pipe was released and the water valve was turned again, the machine was set in 3 minutes with the water running. Then flushing was repeated for 1 minute without running and then 3 minutes with the water running in (3-1-3-1-3). The sieving process was then finished and the cover (top lid) was removed. The sieves without rubber rings were placed into the oven (WTC binder FD-53, Tuttlingen, Germany) for overnight drying at 104°C. After overnight drying, sieves and two trays with samples were taken out and weighed.

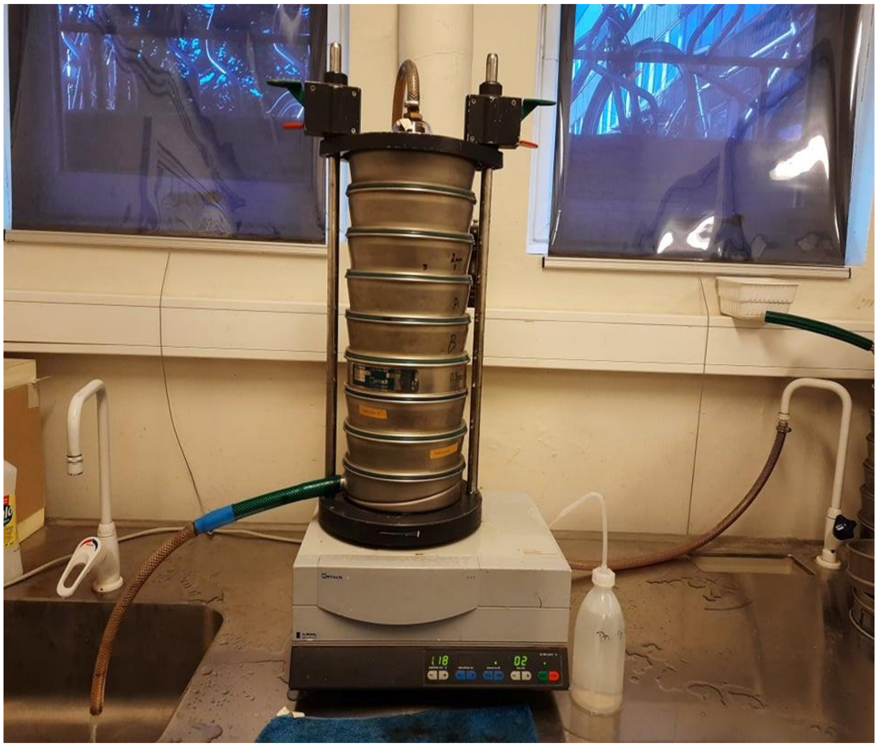

Figure 17. Retsch Sieve shaker fitted with the water inlet and outlet pipe for wet sieving process

2.6.1.3 Laser diffraction method (Mastersizer 2000)

The laser diffraction method was used to determine the size distribution of intestinal contents on a Malvern Mastersizer S instrument (Malvern Instruments Ltd, Worcestershire, UK) as described by (Hetland et al., 2002). 5 to 6 grams of representative samples were taken and dissolved in distilled water and the dissolved samples were dispersed in a particle dispersion unit and circulated through the cell, which was placed in the beam's path. The focal length was 300 mm, and the depth of the cell was 24 mm. Results were displayed in both volume % and D-form (D10, D50, and D90).

D50: Median value (D_{50}) of a volume-based PSD (particle size distribution) is the size in microns at which 50% of the measured sample volume is smaller and 50% is larger (Malvern, 1999). For example, if the median value (D_{50}) of a volume-based PSD is 100 µm, this means that particles with a size up to 100 µm account for 50% of the measured sample volume.

D10: is the size of particle in microns below which 10% of the sample lies.

D90: is the size of particle in microns below which 90% of the sample lies.

2.7 Data analysis

The data were analyzed by one-way ANOVA using SAS and R programming. Performance data were analyzed by using SAS and the rest analysis was done through R. Differences were considered to be significant at $P < 0.05$. When a significant difference was detected, means were separated using the Tukey HSD test. Graphs were created in Microsoft Excel and tables in Microsoft Word.

3. Results

3.1 Test grindings

3.1.1 Cereals geometric dimensions

Table 6. Geometric dimensions of the four major grains ingredient

Cereals	Length	Width	Thickness	Hardness
Maize	11.84	8.50	5.12	20.82
Wheat	6.07	3.36	2.55	12.9
Barley	8.54	3.85	2.95	18.77
Oats	10.46	3.1	2.59	23.92

Maize had the greatest length, width, and thickness among the four major kinds of cereal employed in this experiment (Table 6). Oats had the highest hardness, followed by maize and barley. Among 4 kinds of cereal, all the dimensions were smaller in the case of wheat except for the width.

3.1.2 Grinding capacity and energy consumption

The grinding capacity varies from cereals to cereals as shown in figure 18, where grinding capacity is much higher for maize than for wheat, barley, and oats. The grinding capacity of the oats was increased dramatically by increasing the screen hole diameter from 3 to 8 mm. Less difference was noticed in the case of maize (Figure 19). Also, it was found that switching the hammer mill's 3 mm screen to an 8 mm sieve reduced energy consumption per ton by 43 and 59 percent, respectively (i.e on average about a halving of energy consumption per ton) for maize and wheat. The reduction in energy utilization was much greater for the harder-to-grind hulls-containing cereals barley and oats, at 64 and 78%, respectively (Data not shown in the form of a table).

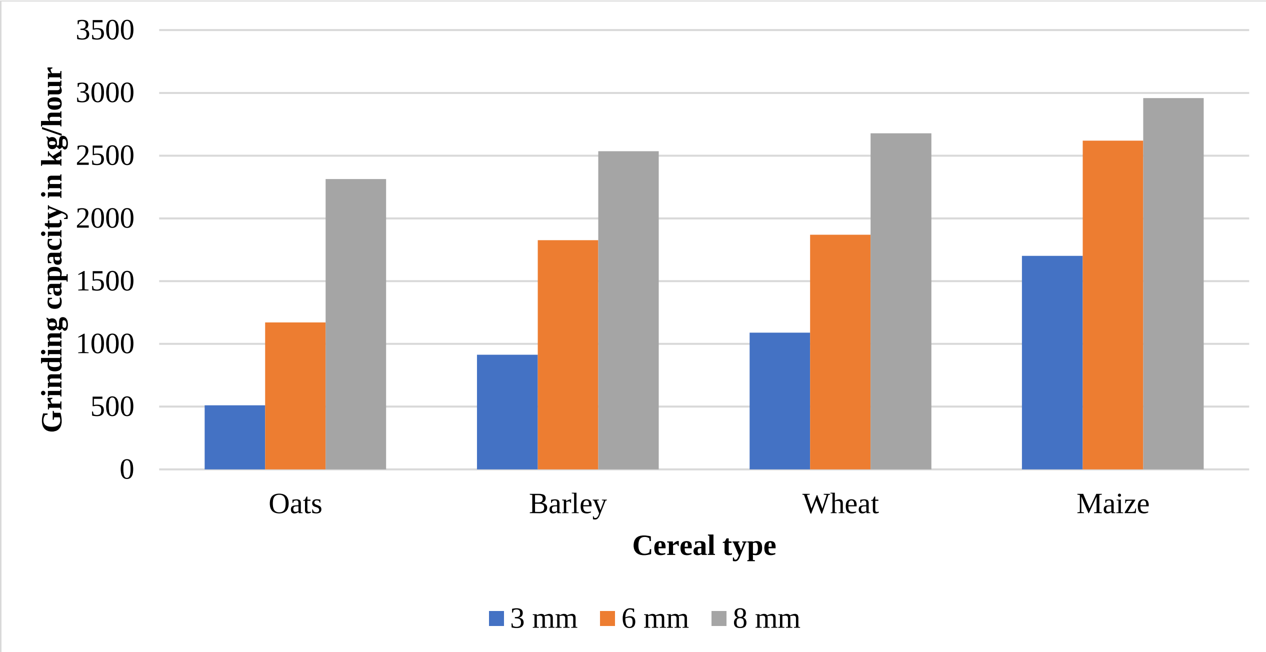

Figure 18. Effect of cereal type and screen hole diameter on grinding capacity of a hammer mill set to a fixed amperage of 18

3.1.3 Cereals particle size analysis

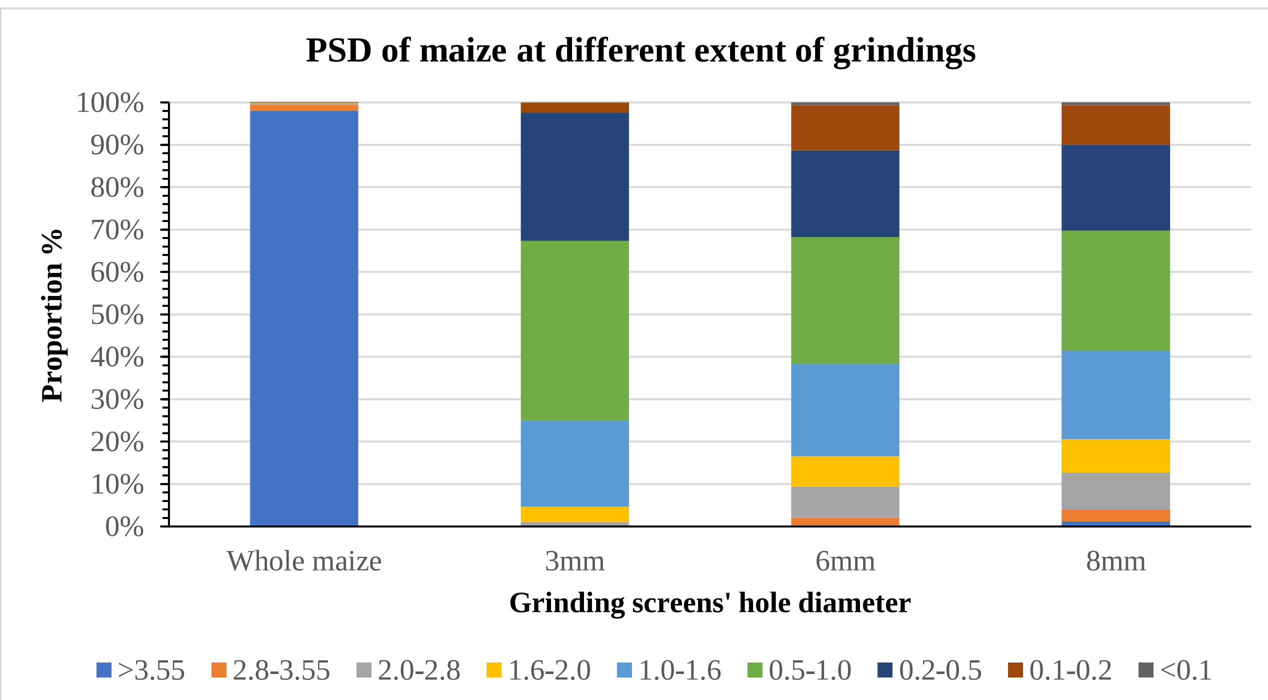

Figure 19. Particle size distribution (PSD) of maize ground at increasing hole diameters.

As shown in figure 19, maize grinding resulted in a majority of the particles being 0.5 mm or greater in size independent of screen pattern. By increasing the hammer mill screen opening diameter, however, the proportion of coarse particles > 1 mm increased, being highest in the 8 mm sieve (24.9%, 38.3%, and 41.3%, respectively for 3, 6, and 8 mm). Wheat, barley, and oats followed a similar pattern, as illustrated in Figures 20, 21, and 22, respectively. The majority of whole oats passed through a 3.55 mm sieve, compared to one-fourth and one-third of wheat and barley, respectively.

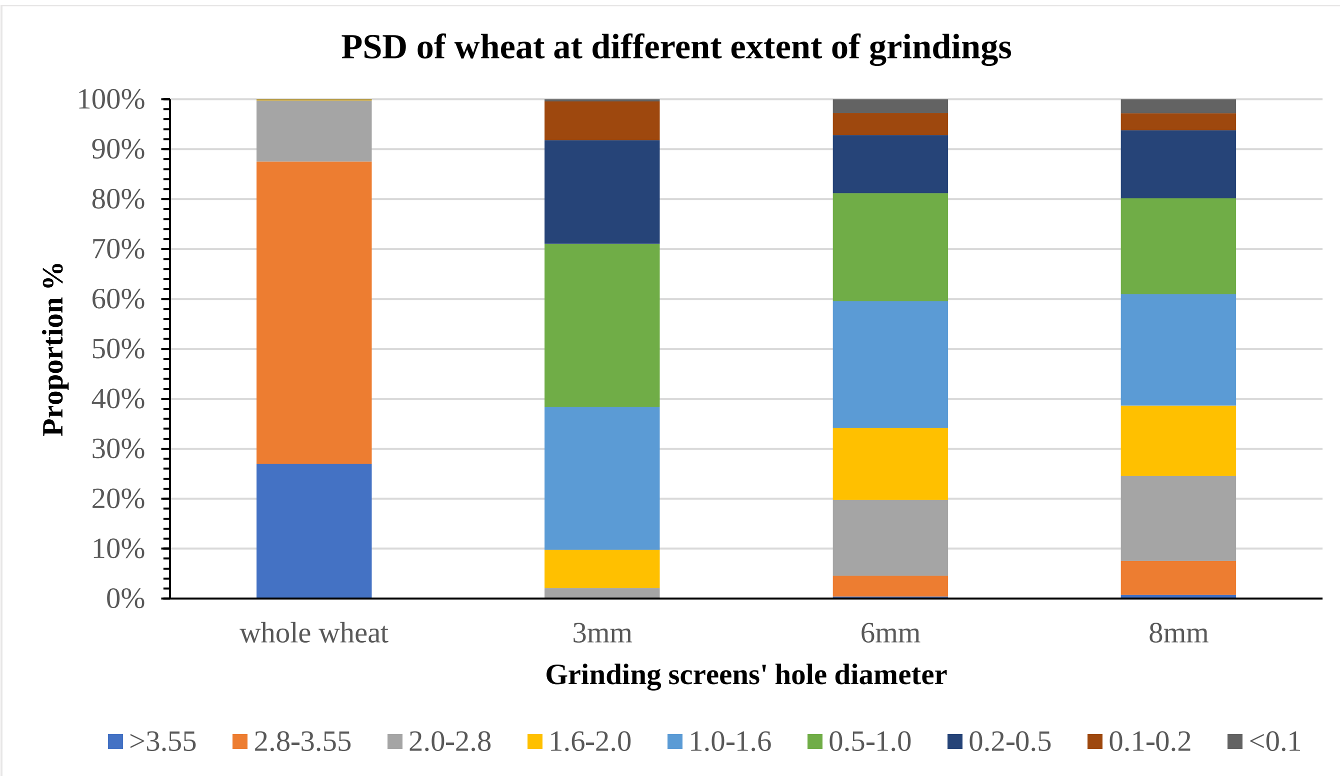

Figure 20. Particle size distribution (PSD) of wheat ground at increasing hole diameters.

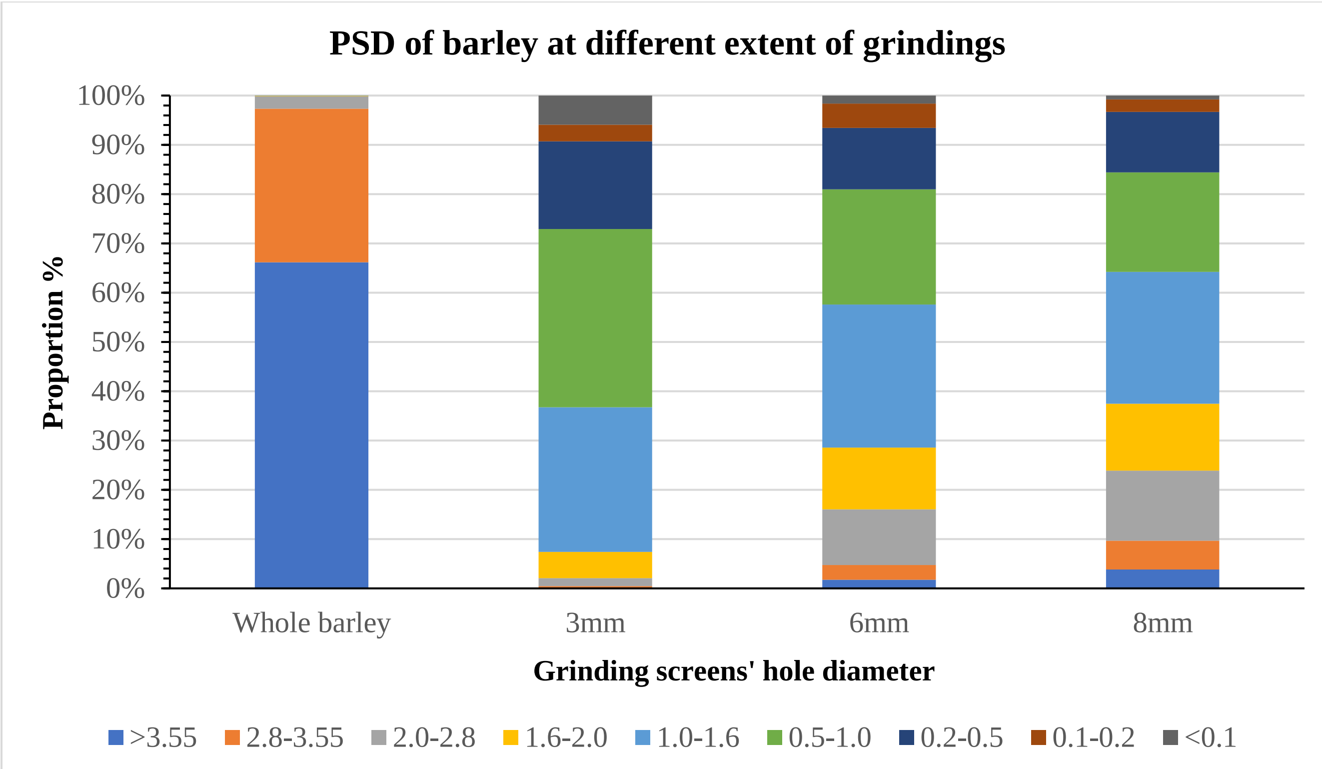

Figure 21. Particle size distribution (PSD) of barley ground at increasing hole diameters.

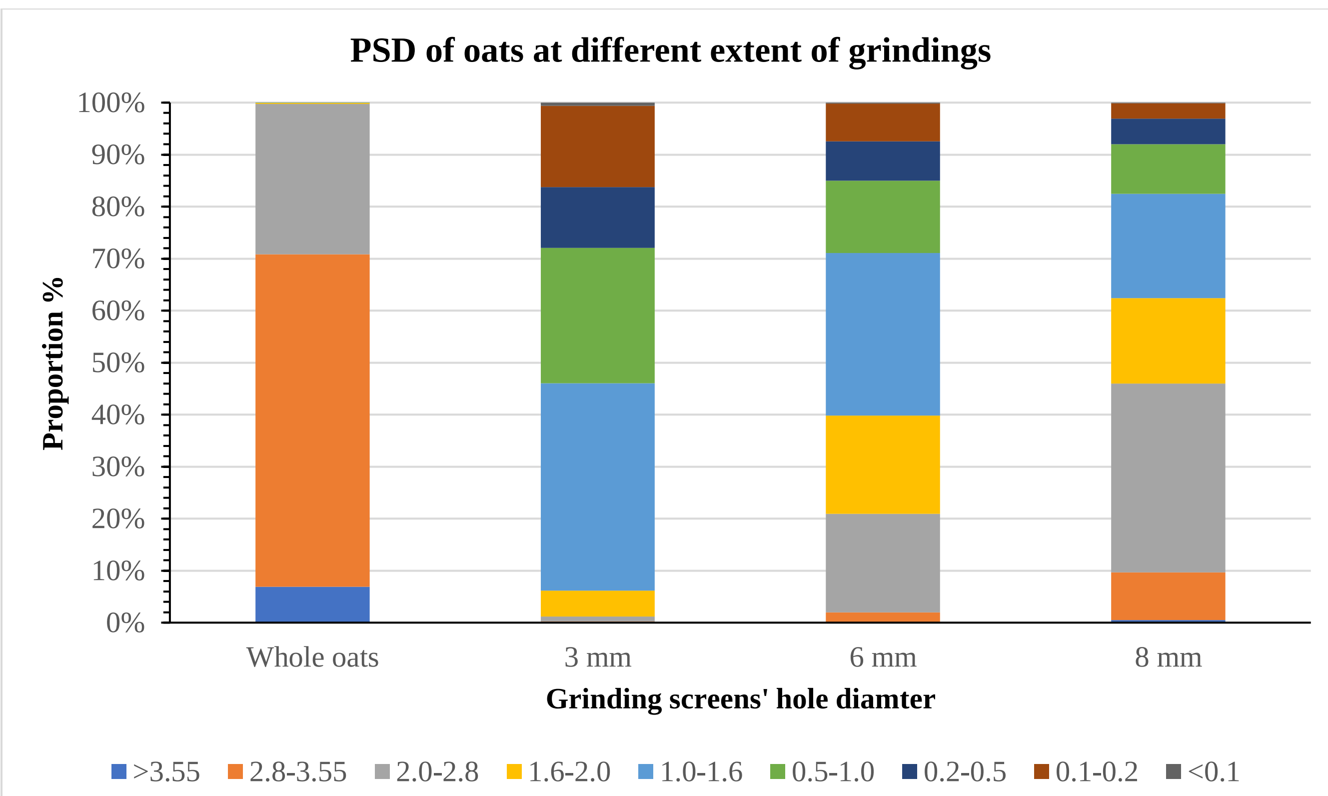

Figure 22. Particle size distribution (PSD) of oats ground at increasing hole diameters.

3.2 Experimental diets and animal experiments

3.2.1 Grinding results

In this experiment, maize and wheat were used as these two kinds of cereal are the principal energy sources used in poultry diets in most countries because of their high-energy value, high protein, etc. One 3 mm normal screen and two novel screens (**A** and **B**) that were used in the test grindings were selected for the processing of experimental diets.

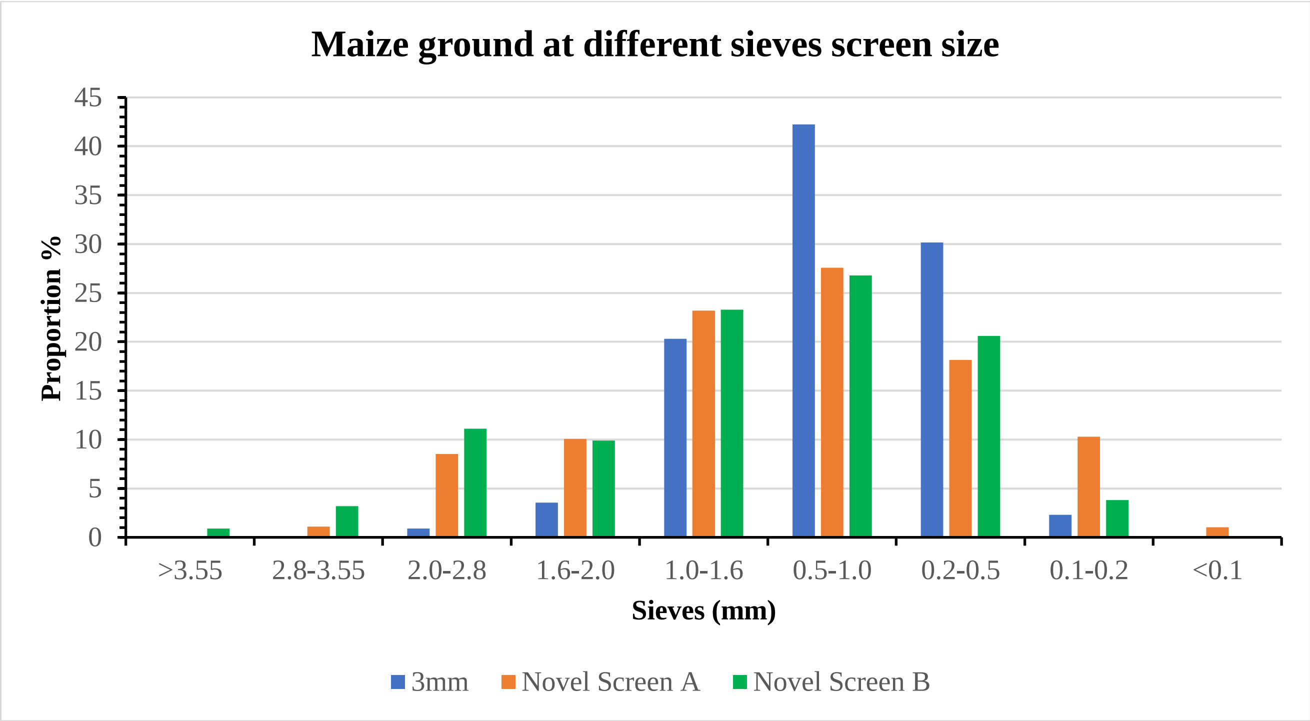

Figure 23. Particle size distribution of the maize ground with 3mm and novel screens.

Novel Screen **A**: Moderately coarse, Novel Screen **B**: Very coarse, and **3mm**: Normal 3mm diameter screen.

The particle size distributions of maize (Figure 23) determined by dry sieving revealed that maize ground using new screens (A and B) had a higher proportion of particles >1 mm than maize ground using a normal 3 mm diameter screen hole. When employing new screens, A and B, the proportion of particles larger than 1mm was 43 % and 48.5 %, respectively, however, when using the 3mm screen, only 23% of particles larger than 1mm were recovered. When ground through a 3mm screen, more fines (< 1mm) were recovered than when ground through screens A and B.

In the case of wheat also, a similar trend was seen (Figure 24). The fraction of particles >1.6 mm ground through screens (A and B) was greater than the standard screen. When using screens A, B, and a standard 3mm diameter screen, approximately 49 percent, 40.5 percent, and 10 percent of the particles were over 1.6 mm, respectively. When employing novel screens, A and B, the proportion of particles larger than 1 mm was 65.1 % and 71.9 %, respectively.

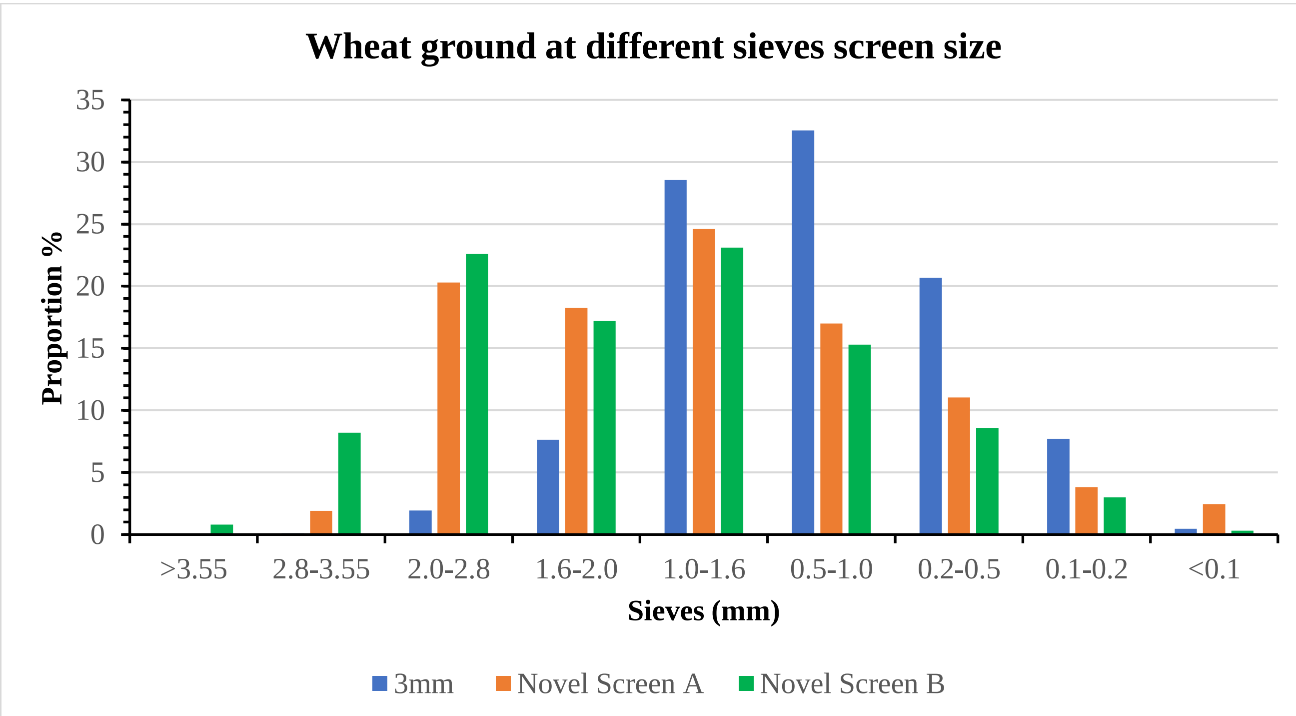

Figure 24. Particle size distribution of the wheat ground at 3mm and novel screens.

Novel Screen **A**: Moderately coarse, Novel Screen **B**: Very coarse, and **3mm**: Normal 3mm diameter screen.

3.2.2 Physical parameters

Table 7. Physical parameters of the pellets used in the experiment

Diet	Length (mm)	Diameter (mm)	Hardness (Kg)	PDI % [a]	Post-pellet temperature (°C)
SP	5.35	3.00	5.17	85.43	82.00
VCP	5.28	3.00	5.67	82.00	81.50
VCPM1	5.60	2.98	6.27	89.37	83.60
VCPM2	5.67	2.98	6.03	92.97	83.00
MCP	5.15	2.99	5.93	82.07	82.00
MCPM1	5.62	2.98	6.63	90.77	82.90
MCPM2	5.41	2.98	6.20	90.73	83.1

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

[a] Pellet Durability Index (PDI); values are means of two replicates of 100±5 g whole pellets.

Pellets produced from cereals that were ground finely, extremely coarsely, and moderately coarsely had lengths and diameters that were almost identical (Table 7). Pellets made from fines of one (diets VCPM1 and MCPM1) and two sievings (diets VCPM2 and MCPM2) of very coarsely and moderately coarsely ground grains had increased hardness and PDI. The post-pelleting temperatures varied only slightly amongst the diets.

3.2.3 Comparison of the particle size distribution of mash and pelleted diets

Wet sieving of mash and pelleted diets (Figure 25) revealed that pelleting reduced the relative proportion of particles > 1 mm and increased the proportion of small particles < 0.1 mm in all three pelleted diets. For diets with extremely coarse grinding (VCP), this effect was particularly pronounced.

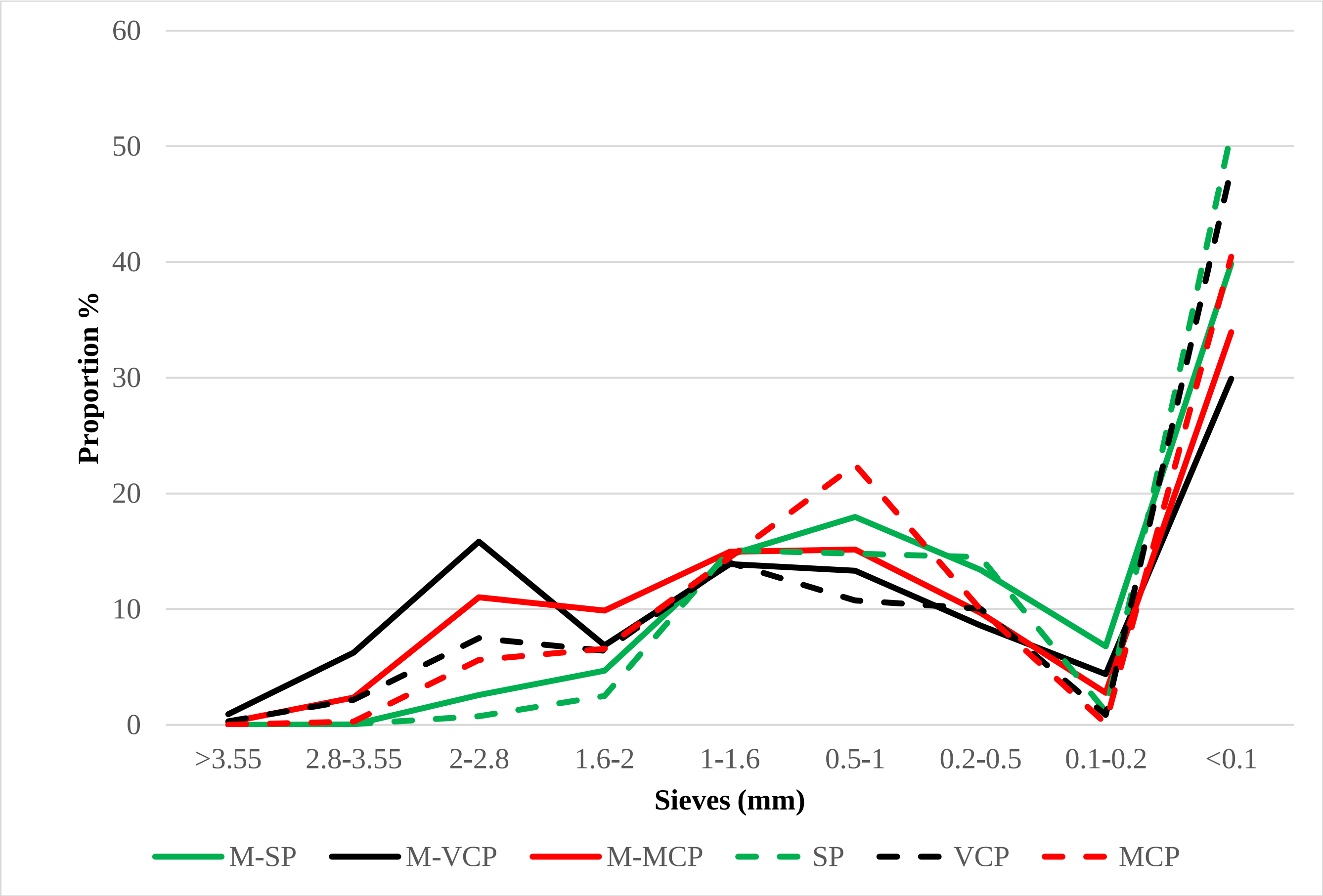

Figure 25. Particle size distribution of mash (solid lines) compared to the same diets after pelleting (dotted lines).

M: Mash, **SP**: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground **P**elleted, and **MCP**: **M**oderately **C**oarsely ground and **P**elleted

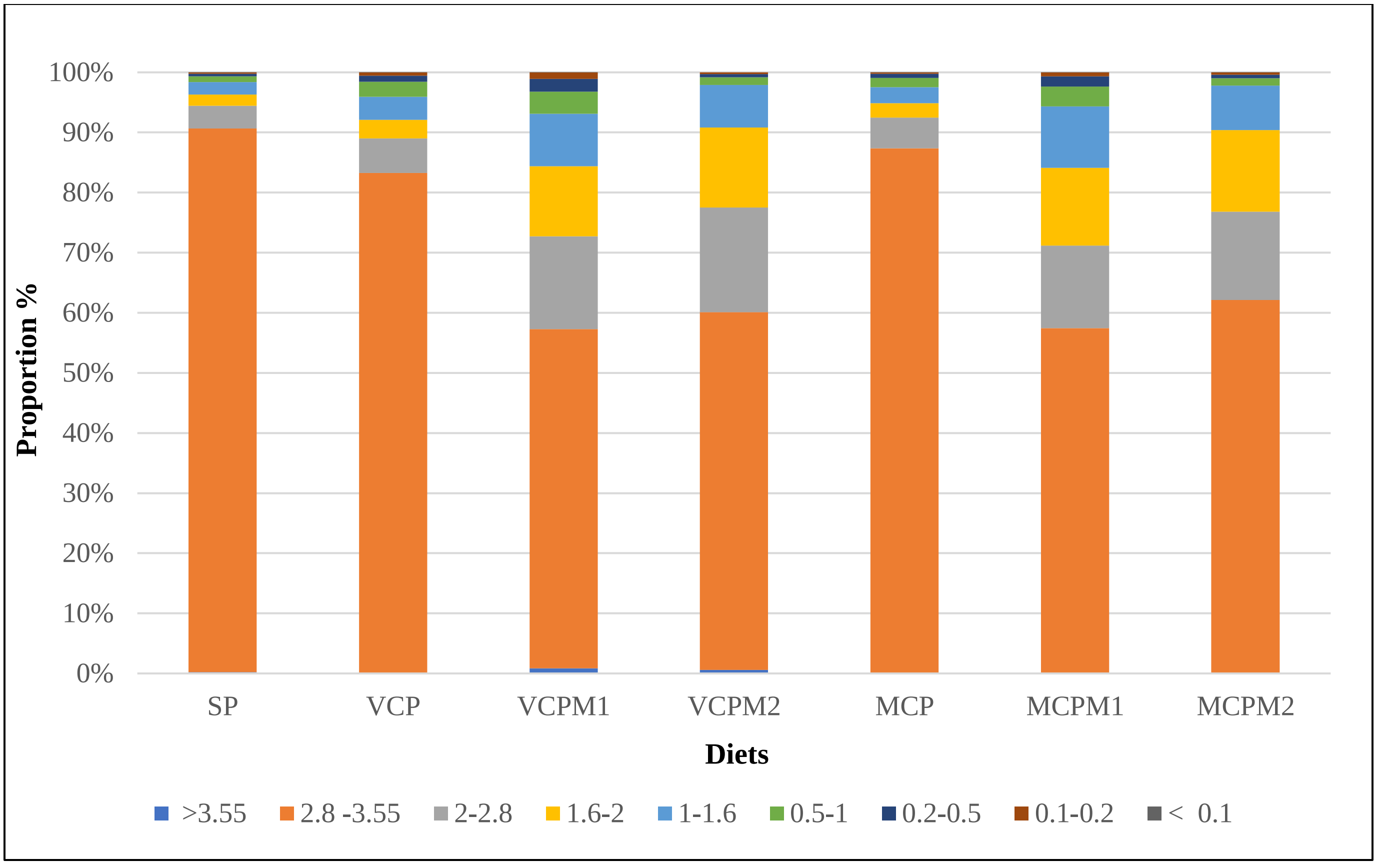

Figure 26. Dry sieving of the seven experimental diets

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

Figure 26 depicts the particle distribution patterns for the experimental diets by feed form as determined by dry sieving. Within these two classes, pelleted diets (SP, VCP, and MCP) and heterogeneous diets (MCPM1, MCPM2, VCPM1, and VCPM2), the particle size distribution was essentially comparable, with just minor differences between them. Wet-sieving analysis of the feed (Figure 27) demonstrated as expected that coarse grinding followed by sieving had a significant impact on the amount of smaller particles size. In these heterogeneous diets, the highest relative fraction of particles > 1 mm was found after double sievings (VCPM2 and MCPM2), accounting for 39.13 percent and 37.69 percent, respectively. The standard pelleted (SP) diet, had the smallest proportion of particles > 1mm (18.38%) and a greater proportion of fine particles (81.62%).

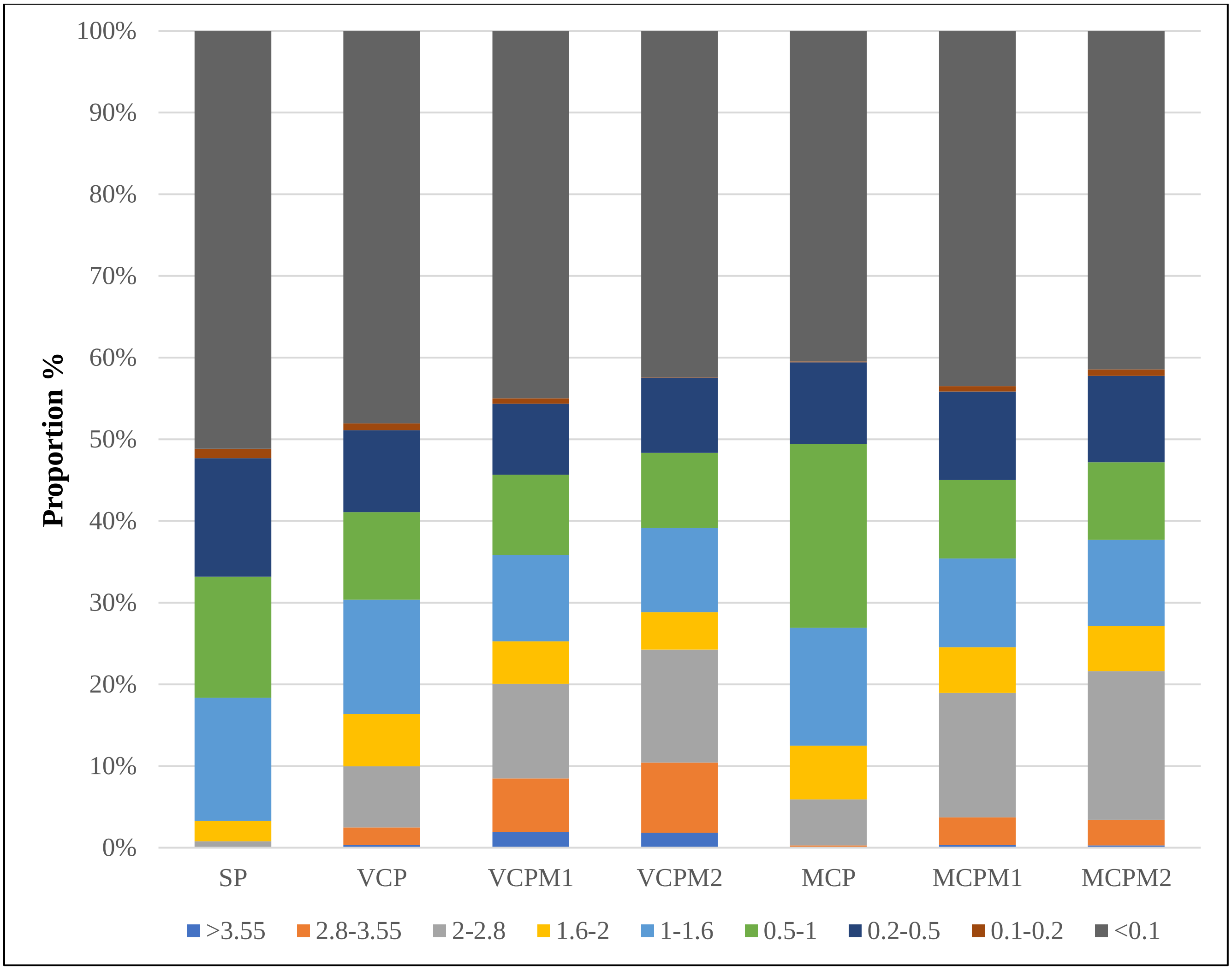

Figure 27. Wet Sieving of the seven experimental diets

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

3.3 Animal experiment results

3.3.1 Growth performance

The coefficient of variation (CV, %) and mortality rates are shown in Table 8. Uniformity was highest (lowest variation) in pelleted diets and lowest (highest variation) in the heterogeneous diet. Within pelleted and heterogenous diets, the percentage was essentially comparable with minimal differences. Mortality during the experiment was negligible (between 1.6 % and 2.9 %) and the deaths were not associated with any specific treatments.

Feed intake and weight gain were influenced ($P < 0.05$) by dietary treatments. Using very or moderately coarse grains in the regular pelleted diets (VCP and MCP), feed intake and body weight gain did not differ ($P > 0.05$) from the standard pelleted diet (SP) (Table 9).

From period 10 to 17 days, while MCPM1 treatment resulted in the lowest feed intake, birds in other dietary treatments gained a similar ($P > 0.05$) intake to those fed the SP except VCPM1 fed birds ($P < 0.05$). The feed intake was lower ($P < 0.05$) in birds fed VCPM1 compared to SP-fed birds. Birds fed SP and MCP diets gained similar ($P > 0.05$) weight to that of diet VCP but higher than other dietary treatments.

During the finishing period (17-31 days), whilst MCPM1 treatment resulted in the lowest feed intake, birds in other dietary treatments gained a similar ($P > 0.05$) intake to the standard pelleted diet (SP). There was no difference ($P > 0.05$) between the weight gain of birds fed the SP diet and other dietary treatments except VCPM1. Birds fed VCPM1 had a lower ($P < 0.05$) gain than treatments SP and MCP.

Throughout the trial (10-31 d) period, it can be seen that, though there was no difference between FI of birds fed the SP diet and other dietary treatments, birds offered the VCP diet consumed more ($P < 0.05$) feed than those fed VCPM1 and MCPM1 diets. In the case of BWG, birds fed heterogeneous diets had lower values ($P < 0.05$) than MCP-fed birds. FCR did not vary ($P > 0.05$) with any of the dietary treatments.

Table 8. Coefficient of variation (CV, %) of slaughtered weight and mortality (%) of broilers fed experimental diets

Diet	CV (%)	Mortality (%)
SP	12.3	2.2
VCP	11.8	2.4
VCPM1	18.2	2.9
VCPM2	16.9	1.8
MCP	12.2	1.6
MCPM1[1]	-	2.2
MCPM2	18.1	2.0

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

[1] CV of diet MCPM1 was missing due to a technical error while taking the weight in the slaughterhouse.

Table 9. Effects of dietary treatments on growth performance from day 10 to 31 days[1]

Diet	Feed intake			Weight gain			FCR[2]		
	10-17 d	**17-31 d**	**10-31 d**	**10-17 d**	**17-31 d**	**10-31 d**	**10-17d**	**17-31d**	**10-31d**
SP	535a	2016ab	2551abc	456a	1471ab	1928ab	1.17	1.37	1.32
VCP	533a	2056a	2589a	454ab	1467abc	1921abc	1.17	1.40	1.34
VCPM1	509bc	1988ab	2496bc	435c	1416c	1851d	1.17	1.40	1.35
VCPM2	522abc	2009ab	2531abc	441bc	1421bc	1862cd	1.18	1.41	1.36
MCP	534a	2041a	2576ab	460a	1476a	1936a	1.16	1.37	1.32
MCPM1	503c	1963b	2467c	434c	1419bc	1854d	1.16	1.38	1.33
MCPM2	527ab	2012ab	2538abc	439c	1432abc	1871bcd	1.20	1.40	1.35
√MSE[3]	17.3	53.1	62.1	11.9	38.5	47.8	0.037	0.032	0.028
***p*-value**	**0.0003**	**0.0121**	**0.0013**	**<0.0001**	**0.0010**	**0.0001**	**0.3181**	**0.1438**	**0.1009**

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

[1]Number of replicates per diet was 9 (nine).

[2]Feed conversion ratio (FCR) corrected for mortality where the weight of the dead birds is included.

[3]√MSE: Square root of mean square error in the analysis of variance

[a-d] Means in a column not sharing a common superscript are different ($P < 0.05$).

3.3.2 Gizzard characteristics

When expressing the empty gizzard weight relative to the percent of body weight, the pelleted-only diets showed significantly lower ($P < 0.05$) weight compared to VCPM1 and VCPM2. Birds fed a standard pelleted diet had the lowest relative gizzard and contents weight compared to the heterogeneous diets. The pH of the gizzard was not ($P > 0.05$) conclusively affected by the dietary treatments (Table 10). On the digestibility of starch and protein, the diets had no significant influence (P > 0.05) (Table 11).

Table 10. Bodyweight and gizzard characteristics on day 32[1]

Diet	Bodyweight d 32	Gizzard				
		pH	Full weight[2]	Empty weight	Relative Gizzard weight[3]	Relative Content weight[4]
SP	2433.00	3.17	30.70b	24.30b	1.00c	0.30b
VCP	2510.90	2.86	44.00ab	26.50ab	1.06bc	0.70ab
VCPM1	2436.55	2.95	47.60a	30.50a	1.26a	0.71a
VCPM2	2451.11	2.78	52.40a	31.00a	1.27a	0.87a
MCP	2526.44	3.17	43.00ab	26.80ab	1.06bc	0.64ab

MCPM1	2537.77	2.83	53.30a	30.40a	1.21ab	0.88a
MCPM2	2432.33	3.03	47.70a	28.90ab	1.20ab	0.77a
√MSE[5]	216.78	0.54	8.74	4.01	0.17	0.27
***p*-value**	**0.887**	**0.643**	**<0.001**	**0.008**	**0.012**	**<0.001**

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

[1] Number of replicates per diet was 9.

[2] Full gizzard weight: Gizzard with contents but without per-gizzard fat.

[3] Empty gizzard weight expressed in relative to % of body weight.

[4] Gizzard content DM weight expressed in relative to % of body weight.

[5]√MSE: Square root of mean square error in the analysis of variance.

[a-b] Means in a column not sharing a common superscript are different ($P < 0.05$).

Table 11. Ileal starch and protein digestibility on day 31[1]

Diet	Starch digestibility (%)	Protein digestibility (%)
SP	97.5	83.5
VCP	96.8	87.4
VCPM1	98.2	86.0
VCPM2	98.5	88.3
MCP	98.4	85.5
MCPM1	98.5	85.9
MCPM2	97.6	85.6
***p*-value**	**0.1710**	**0.1500**

√MSE[2]	1.44	3.08

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

[1]Number of replicates per diet was 9.

[2]√MSE: Square root of mean square error in the analysis of variance.

3.3.3 Gizzard particle size analysis

The inclusion of moderately coarsely ground cereals with pellets in the diet, MCPM1, resulted in a higher proportion of particles in the gizzard contents with a size > 1.6 mm (Table 12). Particles > 2.8 were similar in all diets when compared to the SP diet but birds fed MCP had a lower proportion ($P < 0.05$) than those fed MCPMP1. Birds fed SP diet had a lower proportion of particle sizes between 1 - 1.6 mm and 1.6 - 2 mm MCP which had similar particles of 1.6 mm- 2 mm, however, no difference ($P > 0.05$) was observed in MCP and MCPM1 fed broilers. No significant differences in particle sizes from 0.5 to 1 mm were observed between the three treatments. Birds on the MCPM1 treatment had the lowest ($P < 0.05$) proportion of particles < 0.5 mm.

While comparing the particle size of gizzard contents with the value of the diets, the proportion of particles > 2.8 mm and < 0.5 mm was much higher and lower in the gizzard content, respectively.

Table 12. Particle size distribution (calculated as % of dry matter) of gizzard contents

Diet	>2.8 mm	2-2.8 mm	1.6-2 mm	1-1.6 mm	0.5-1 mm	<0.5 mm
SP	10.8 ab	4.3 b	2.8 b	7.9 b	17.0	57.2 a
MCP	4.7 b	6.5 b	8.3 ab	15.5 a	16.9	48.1 a
MCPM1	14.5 a	13.8 a	12.4 a	14.4 a	14.2	30.7 b
***p*-value**	**0.044**	**0.012**	**0.021**	**0.011**	**0.425**	**0.012**
√MSE[1]	1.521	0.964	1.143	0.780	1.438	2.625

SP: **S**tandard **P**elleted, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving

[1]√MSE: Square root of mean square error in the analysis of variance.

[a-b] Means in a column not sharing a common superscript are different ($P < 0.05$)

When compared to the standard pelleted diet, birds fed the MCP diet and MCPM1 tended ($P = 0.057$) to have the lowest proportion of litter in the gizzard (Table 13). The weight of litter retained in the sieves was not significantly different ($P > 0.05$) between any of the dietary treatments examined. The weight of the litter retained in the >2.8 mm sieve was higher overall than the weight of the liter retained in the other sieves.

Table 13. Weight and % of litter retained from gizzard DM contents

Diet	**Dried litter weight retained (gram)**					
	>2.8 mm	**2-2.8 mm**	**1.6-2 mm**	**1-1.6 mm**	**Total weight of litter**	**Litter, %[1]**
SP	0.78	0.34	0.12	0.06	1.30	16.1
MCP	0.95	0.48	0.11	0.04	1.57	7.24
MCPM1	1.62	0.31	0.11	0.03	2.06	7.16
***p*-value**	**0.305**	**0.859**	**0.995**	**0.622**	**0.693**	**0.057**
√MSE[2]	0.3303	0.2229	0.0602	0.0189	0.6020	1.7486

SP: **S**tandard **P**elleted, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCMP1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving

[1]Litter (%): (Sum weight of litter / Dry matter weight of initial sample) * 100,

[2]√MSE: Square root of mean square error in the analysis of variance.

3.3.4 Feeding behavior

Table 14. Percent of birds observed eating, the sum for 4 - time points during 4 minutes on day 20[1]

Diet	% Of birds eating[2]
MCP	40.31
MCPM1	42.99
MCPM2	45.46
***p*-value**	**0.750**
√MSE [3]	13.4719

MCP: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**1**) sieving.

[1]Number of replicates per diet was 9.

[2]Mortality during the data collection day was corrected.

[3]√MSE: Square root of mean square error in the analysis of variance.

Despite a numerical increase in eating time with increased coarseness, no significant differences were found in eating time (Table 14).

Tables 15 and 16 show the dry sieving particle size distribution pattern of feed collected every hour for four hours from the feeders of birds fed VCPM1 and MCPM2 diets, respectively. The fraction of particles > 2.8 mm was similar ($P > 0.05$) from the first to the last hour of feed accessed but was lower ($P < 0.05$) than time zero (before birds' access to the feed) in VCPM1. Although there was no difference in particle sizes between time 1 and time 2 hours and other times (0, 3, and 4 hours) in birds fed the MCPM2 diet, a smaller proportion ($P < 0.05$) of particles >2.8 mm was seen at times three and four than at time zero. While no significant difference was seen in the range of particles from 1.6 mm and 2 mm when fed the MCPM2 diet, the fraction of particles in 1.6 and 2 mm sieves in diet VCPM1 was comparable ($P > 0.05$) from time 1 to time 4 but higher than time zero. In time zero a lower percentage of 1 mm-diameter particle were seen than in time 2, 3, and

4 hours (in VCPM1) and time 3 and 4 hrs (in MCPM2). Particles ≤ 1 mm in both diets did not change significantly with time.

Table 15. Particle size distribution of the feed collected every hour for 4 hours, diet **VCPM1**[1]

Time (hr.)	**>2.8 mm**	**2-2.8mm**	**1.6-2mm**	**1-1.6mm**	**0.5-1mm**	**<0.5mm**
0	61.2 a	14.7 b	10.7 b	7.9 b	3.3	2.2
1	48.7 b	18.5 a	15.1 a	11.3 ab	3.95	2.42
2	41.5 b	18.5 a	16.9 a	14.3 a	5.73	3.07
3	39.4 b	18.3 a	17.3 a	15.2 a	6.34	3.44
4	43.2 b	18.2 a	16.8 a	14.2 a	5.10	2.49
***p*-value**	**<0.001**	**0.002**	**<0.001**	**0.002**	**0.104**	**0.444**
√MSE[2]	4.803	1.081	1.205	1.962	1.416	0.957

[1] **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving

[2]√MSE: Square root of mean square error in the analysis of variance.

[a-b] Means in a column not sharing a common superscript are different ($P < 0.05$)

Table 16. Particle size distribution of the feed collected every hour for 4 hours, diet **MCPM2**[1]

Time (hr.)	**>2.8 mm**	**2-2.8mm**	**1.6-2mm**	**1-1.6mm**	**0.5-1mm**	**<0.5mm**
0	58.6 a	14.7	13.6	9.74 b	1.90	1.44
1	52.3 ab	15.3	16.2	11.3 ab	2.51	2.03
2	50.3 ab	15.2	16.5	12.2 ab	3.14	2.44
3	47.3 b	14.6	17.8	13.9 a	3.43	2.75
4	45.5 b	14.8	17.9	14.5 a	3.93	3.25
***p*-value**	**0.023**	**0.984**	**0.363**	**0.046**	**0.877**	**0.916**
√MSE[2]	4.450	1.924	2.779	1.896	2.532	2.480

[1] **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**1**) sieving.

[2]√MSE: Square root of mean square error in the analysis of variance.

[a-b]Means in a column not sharing a common superscript are different ($P < 0.05$).

3.3.5 Particle size analysis of the intestinal and excreta contents

When expressed as D10, D50, and D90, the particle size distribution of the duodenal, jejunal, and ileal contents did not significantly differ (P > 0.05) among the diets (Table 17).

Table 17. Particle size distribution of small intestinal content in µm

Diet	D10			D50			D90		
	Duo	**Jej**	**Ile**	**Duo**	**Jej**	**Ile**	**Duo**	**Jej**	**Ile**
SP	16.1	24.6	58.6	192.0	246.0	359.1	751.0	870.0	978.7
VCP	17.6	20.8	48.7	160.0	202.0	307.0	657.0	841.0	964.9
MCP	17.7	25.4	57.3	158.0	205.0	336.0	594.0	841.0	928.3
***p*-value**	**0.981**	**0.743**	**0.360**	**0.716**	**0.238**	**0.518**	**0.194**	**0.576**	**0.806**
√MSE[1]	18.681	12.688	16.407	93.017	55.595	92.404	168.262	64.056	146.701

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **MCP**: **M**oderately **C**oarsely ground and **P**elleted. **Duo**: Duodenum, **Jej**: jejunum, and **Ile**: Ileum.

[1]√MSE: Square root of mean square error in the analysis of variance.

3.3.5.1 Duodenum

Except for particles between 0.1 and 0.2 mm ($P < 0.05$), there was no significant difference in particle sizes ($P > 0.05$) as shown in table 18. The particle sizes (0.1-0.2 mm) in the duodenal contents of birds given the MCP and VCP diets were similar ($P > 0.05$) and were higher ($P < 0.05$) than the standard pelleted diet (SP).

Table 18. Particle size distribution (in calculated volume %) of duodenal contents

Diet	1.6-2 mm	1-1.6 mm	0.5-1 mm	0.2-0.5 mm	0.1-0.2 mm	0-0.1 mm
SP	0.2	4.1	19.5	22.4	13.0 b	40.8
VCP	0.1	2.8	15.3	23.0	16.2 a	42.6
MCP	0.1	1.7	13.0	25.4	17.3 a	42.5
***p*-value**	**0.701**	**0.330**	**0.184**	**0.209**	**0.003**	**0.922**
√MSE[1]	0.185	2.9591	6.977	3.374	2.310	10.102

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **MCP**: **M**oderately **C**oarsely ground and **P**elleted.

[1]√MSE: Square root of mean square error in the analysis of variance.

[a-b] Means in a column not sharing a common superscript are different ($P < 0.05$).

3.3.5.2 Jejunum

The particle size distribution of the jejunal contents in terms of computed volume percent (Table 19) showed no significant difference ($P > 0.05$) between the diets.

Table 19. Particle size distribution (in calculated volume %) of upper jejunal contents

Diet	1.6-2 mm	1-1.6 mm	0.5-1 mm	0.2-0.5 mm	0.1-0.2 mm	0-0.1 mm
SP	0.3	6.1	23.2	23.3	15.3	31.8
VCP	0.3	5.6	20.3	22.2	16.4	35.2
MCP	0.3	5.7	20.5	22.5	16.7	34.3
***p*-value**	**0.968**	**0.623**	**0.168**	**0.647**	**0.052**	**0.442**
√MSE[1]	0.11	1.302	3.266	2.409	1.136	5.508

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **MCP**: **M**oderately **C**oarsely ground and **P**elleted.

[1]√MSE: Square root of mean square error in the analysis of variance.

3.3.5.3 Ileum

None of the dietary interventions affected the particle size distribution of lower ileal contents (P > 0.05) (Table 20).

Table 20. Particle size distribution (in calculated volume %) of lower ileal contents

Diet	1.6-2 mm	1-1.6 mm	0.5-1 mm	0.2-0.5 mm	0.1-0.2 mm	0-0.1 mm
SP	0.7	9.3	27.5	26.4	14.0	22.1
VCP	0.6	8.4	24.4	25.8	16.5	24.3
VCPM1	0.8	10.4	26.8	26.8	15.6	19.6
VCPM2	0.5	7.4	24.1	28.0	16.8	23.2
MCP	0.5	8.7	24.7	26.9	15.9	23.3
MCPM1	0.7	9.3	27.1	28.1	15.6	19.2
MCPM2	0.6	9.9	28.0	27.9	15.1	18.5
***p*-value**	**0.799**	**0.791**	**0.526**	**0.416**	**0.293**	**0.541**
√MSE[1]	0.43	3.83	4.92	2.49	2.31	7.15

SP: **S**tandard **P**elleted, **VCP**: **V**ery **C**oarsely ground and **P**elleted, **VCPM1**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving, **MCP**: **M**oderately **C**oarsely ground and **P**elleted, **MCPM1**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after one (**1**) sieving, and **MCPM2**: **M**oderately **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

[1]√MSE: Square root of mean square error in the analysis of variance.

3.3.5.4 Excreta

There were no significant differences (*P* > 0.05) between the two diets in the size of particles retained in the sieves (2 mm, 1 mm, 0.5 mm, and 0.5 mm) from excreta collected (Table 21).

Table 21. Particle size distribution of the excreta contents

Diet	>2 mm	1-2 mm	0.5-1 mm	<0.5 mm
VCP	2.4	8.7	16.3	72.6
VCPM2	3.0	9.9	14.3	72.8
***p*-value**	**0.743**	**0.419**	**0.262**	**0.934**

$\sqrt{MSE}$[1]	1.690	1.626	1.901	4.185

VCP: **V**ery **C**oarsely ground and **P**elleted, **VCPM2**: **V**ery **C**oarse cereal **M**ixed with **P**ellets after two (**2**) sieving.

[1]$\sqrt{MSE}$: Square root of mean square error in the analysis of variance.

4. Discussion

4.1 Grinding and experimental diets' processing part

Different types of pilot grinding and sieving trials were first conducted at the Center for feed Technology in order to understand the effect of screen choice on the particle size distribution of different cereals. The goal of the test grinding was to obtain coarse microstructure while examining the impact of hammer mill screen opening diameters on particle sizes. The two advantages of coarser grinding are that it boosts gizzard function and improves production efficiency. Since pelleting reduces particle size, as mentioned in the literature review, ground cereals were first sieved in two different levels (one and two sievings), and the resulting fines were then used for pellets production. This preserved the coarse structure from grinding in the pellet press. Test grinding results showed that the relative proportion of ground cereals fraction increased with the increasing diameter of the hammer mill screen. As hammer mill screen opening (3 mm, 6 mm, and 8 mm diameter) increased, the relative proportion of cereals particles > 1 mm also increased. These grindings data support earlier observations where the relative proportion of particles larger than 1 mm increased as the hammer mill screen opening diameter increased (Amerah et al., 2008a; Naderinejad et al., 2016; Svihus, Kløvstad, et al., 2004). Additionally, increasing the diameter of the screen enhanced grinding capacity and decreased energy use (Figure 18) which is in accordance with the findings of Reece et al. (1986) and Vukmirović et al. (2016). The grinding capacity was much higher for maize than for wheat, barley, and oats (Figure 18). The main cause of this variation is different fiber content because fiber-rich cereals are ductile and challenging to ground. This confirms that coarser grinding with an increasing hammer mill screen is efficient from a manufacturing standpoint, as it allows the material to pass easier, and reduces the amount of fines and energy use, which eventually lowers production costs.

When corn and wheat were processed in the same hammer mill with the same novel screen size (A and B), particle size analysis of the ground grains revealed discrepancies in the particle size distribution between the two kinds of cereal. When compared to grinding corn, wheat produced coarser particles greater than 1mm which is consistent with the findings of Amerah et al. (2008a). Nir et al. (1995) also demonstrated that when grinding wheat using the same mill under the same circumstances, the particles were coarser than when grinding sorghum. Because grain hardness affects the particle size of milled grains, these results may be related to variations in endosperm hardness between the grain types (Dobraszczyk et al., 2002). In addition, this study shows that cereals like barley, oats, and wheat with widths between 3.1 and 3.85 mm and thicknesses between 2.55 to 2.59 mm may pass through the holes unbroken and affect the particle size distribution results when hammer screens with bigger diameters are used.

As discussed in the literature review, the possible effect of particle size on pellet durability is contradictory. In this study, the durability of the pelleted-only diets made from coarser grindings (VCP and MCP) were almost similar to those of pellets made from fine grinding (SP). In contrast to medium or fine grindings, Naderinejad et al. (2016) found that maize ground more coarsely had no detrimental effects on pellet durability. It can be concluded that even with coarser grinding pellet quality may not be poor. The lack of negative effects of coarser grinding is surprising, as it has been stated before that coarser grinding may cause weak spots in the pellets and thus reduce the pellet durability (Svihus, Kløvstad, et al., 2004; Thomas et al., 1998). Also, wider particle distribution leads to more inhomogeneities in pellet structure, particularly the presence of more coarse particles, which may be the cause of the lower pellet durability index. Pellets are notably more sensitive close to the locations of structural inhomogeneities because there are more local stresses and strains there (Thomas et al., 1998). Therefore, while using coarser grinding, it is crucial to monitor pellet quality. From the perspective of handling and transportation in the factory and on the farm, the manufacture of high-quality pellets is crucial. Additionally, pellets with superior physical quality will have higher nutritional value (Thomas & Van der Poel, 1996).

The durability index of the pellets within heterogeneous diets (VCPM1, VCPM2, MCPM1, and MCPM2) was better than that of solely pelleted diets (SP, VCP, and MCP). Reimer (1992) asserts that a number of variables, including feed formulation (40 %), particle size (20 %), conditioning (20 %), pellet-mill die specifications (15 %), pellet cooling and drying (5 %), affect the physical quality of pelleted feeds. In the current study, the variation in the composition of pellets between the diets may potentially contribute to the varying different pellet durability indexes. Pellets in the heterogenous diets were made from the fines remaining after separating the coarse cereals. Due to the presence of fine particles, pellet quality was improved because fines have more inter-contact sites, which facilitates the penetration of heat and moisture into feed components which the chemical functions. As a result of increasing chemical alteration of the feed components, such as starch and/or proteins, the binding of the feed particles in the pellets increases and eventually may improve the quality (Zimonja et al., 2008). However, particle size is not the only factor that influenced the pellet quality as discussed earlier. The variation in the conditioning temperature might also be another factor responsible for differences in durability index between the diets (pellets in the heterogeneous and only pelleted diets) In this study, the conditioning temperature of the pellets in the heterogenous diets was 82°C as opposed to 75°C for the diets that contained only pellets. Therefore, the change in pellet durability index between diets can be explained by the difference in conditioning temperatures between pellets of heterogeneous and solely pelleted diets. Netto et al. (2019), observed an increase in the pellet quality from 85.4 to 91.4 % when the conditioning temperature increased from 70 to 90°C. Similarly, Loar II et al. (2014) reported an improvement in pellet durability index while increasing the conditioning temperature from 74 to 85°C. According to Froetschner (2006), the ability of water introduced as steam to agglutinate pellet particles may be the cause of the improvement in pellet quality observed when conditioning temperature and steam rose.

From the results of the pellet durability index (PDI), it can be concluded that it is beneficial to grind the cereals coarsely as coarseness did not impair pellet durability. Coarse particles can be blended with the pellets later on in the appropriate mixture to preserve the larger structure in the diet without subjecting to the pelleting process. This is supported by the wet sieving results, where up to 40% of the coarse particles greater than 1 mm were preserved.

In the current study, it was found that pelleting process reduced the proportion of coarse particles. Particle analysis of diets revealed that the distinctions of the particle size distribution of the coarsely and normal grindings became less noticeable after pelleting. These findings are in accordance with the previous results of Amerah, Ravindran, et al. (2007b); Engberg et al. (2002); Svihus, Kløvstad, et al. (2004). Due to the narrow distance between the rollers and dies during pelleting the coarser particles are particularly prone to grinding and this may explain why the pelleting process even out differences in particle size distribution, hence the reduction in the proportion of larger particles was not unexpected. Abdollahi et al. (2011) assumed that frictional force inside the die holes can further grind coarse particles. By increasing the roller-die gap it is possible to save the coarse microstructure in the pelleted diets. According to the results of the optimization process, Vukmirović et al. (2017) found that the coarsest grinding on a hammer mill (9 mm screen hole diameter) and the largest roller-die gap (2 mm) should be used in order to create pellets of adequate quality with the least amount of energy consumption of the pellet press. Therefore, either widening the roller die gap or omitting the coarse microstructure from pelleting could be the best way to preserve the coarse particles in the poultry diets.

Dry sieving is done to analyze the particle size distribution of the microstructure in the mash diet. When diets are pelleted, dry sieving will not give a measure of microstructure, while complicated and time-consuming wet sieving is performed to know the distribution of the particles in the pelleted diets. The process of wet sieving may very well be a reflection of the pellets disintegration that takes place in the poultry's gastrointestinal tract (mainly in the crop), making it a more accurate and digestion-related illustration of particle size distribution than dry sieving (Hafeez et al., 2015). Wet sieving of finished experimental diets showed that approximately 40% of the particles were greater than 1 mm in VCPM2 and MCPM2 diets. This shows that double sieving of either moderately or coarsely ground cereals using a 2 mm sieve was effective to retain the coarse structure which could be added post-pelleting. Additionally, these particles have a significant impact on how well the digestive tract functions, mostly through their gizzard stimulating properties.

From the feed manufacturing point of view, this study demonstrates that maintaining the coarseness in poultry diets with about 40% of the particles more than 1 mm can be obtained by innovative grindings followed by twofold sieving and separation of the coarse particles without subjecting them to the pelleting process. By doing so it is also possible to preserve the pellet durability which would have been expected to increase the feed intake and gizzard functionality. From an economical aspect, less consumption of energy with the coarser grindings also reduces the production costs.

4.2 Animal experiment part

4.2.1 Broilers performance

The highest feed intake can be attained by feeding the pellets, even if coarse grinding produces large particles that result in a larger feed intake than fine grinding in mash diets. In this experiment, birds fed the VCP diet had higher ($P < 0.05$) feed intake and weight gain than birds fed VCPM1 and MCPM1 (10-31 d). The significant difference shows that when given a heterogeneous diet, there was a tendency for lower feed intake and weight gain. The performance data as a whole also shows that the heterogeneous structure is not able to support the maximal feed intake and weight gain compared to the pelleted diets. Due to the uniqueness in the diet composition, pelleted and heterogenous feeds have different particle size distributions. Also, three pelleted diets had comparable macrostructures, whereas four heterogeneous diets had diverse macrostructures. Heterogeneous diets were a mixture of pellets larger than 3 mm and coarse structures between 1 mm and 2 mm in size, as can be observed in the results of dry sieving. As discussed in the literature review, the preference for larger particles increased with age. So there is a need for bigger particles for better performance, as Portella et al. (1988) found that broilers prefer particles larger than 2.36 mm as broiler aged. Similarly, the feed preference trials in this experiment also support this theory that particle size preference was over 2.8 mm when offered heterogenous diets, which in this case were the pellets. Thus it appears that the coarse particles in the heterogeneous diets were smaller and not preferred by the birds which ultimately affected the performance. It was also observed that (data not shown) the proportion of the weight of both very large and very small birds was higher for the birds given heterogeneous diets compared to the pelleted diets. This is because pellets have a higher nutritional composition than other coarse particles in the diets, therefore dominating birds that might have eaten all the pelleted first would gain more weight than other birds within the same treatment. This may have resulted in a systematic imbalance in nutrient intake affecting the coefficient of variation. It can also be speculated that a high coefficient of variation might have affected the weight gain to some extent. So, unbalanced treatments might be the limitation of this experiment which is also supported by the results of the coefficient of variation between pelleted and heterogeneous diets.

Similarly, in the initial period (10- 17 d), MCPM1-fed birds often showed reduced feed intake than pelleted (SP, VCP, MCP) and MCPM2-fed birds. The reason behind the lower intake in MCPM1-fed birds might be due to the higher amount of fines than MCPM2 and pelleted diets. During the processing of the diets, more fines were removed from MCPM2 as it is a diet with a mixture of pellet and moderately coarse particles after two sievings. And it is also clear from the dry sieving data of the heterogeneous diets that the percentage of fines was higher after one sieving than after two sieving in both moderately coarse (MCPM1 and MCPM2) and very coarse diets (VCPM1 and VCPM2). Also, a similar tendency for feed intake was seen in very coarse and moderately coarse cereals-fed birds where double sieving (VCPM2 and MCPM2) had higher feed intake than single sieving (VCPM1 and MCPM1) in all age groups, though there were no significant differences. This seems to be an indication that reducing the amount of fines or increasing the proportion of coarse particles will have a small positive effect on feed intake compared to the diets with more fines. Many kinds of research showed the negative effect of fines percentages in the pelleted diets or the effect of mixing pellets to the mash diets on the broiler performance. Corzo et al. (2011) observed reduced feed consumption and body weight when birds fed mash diets were compared with diets containing either 32 % pellets or 64% pellets. When compared to the day before the transition, when birds were fed the pelleted diet, the transition to a 50:50 percent mix (pellet: mash) caused a reduction in feed intake (Quentin et al., 2004). Proudfoot and Sefton (1978) and Proudfoot and Hulan (1982) also found a reduced broiler and turkey performance as the proportion of fines in their pelleted feeds increased. These results illustrated the importance of particle size and prehension capacity of the chick. As birds have a preference for larger feed particles in accordance with the size of the beak and oral cavity (Schiffman, 1968), they prefer diets with fewer fines than diets with more fines. Hetland et al. (2002) observed reduced feed intake when fed broiler chickens whole cereals mixed with pellets which could be due to the limited capacity of the gizzard for grinding cereals, followed by a slower passage through the digestive tract. But in our experiment pellets were mixed with coarse cereals of different coarseness, which leads to the difference in feed preferences and ultimately the difference in feed intake.

Similarly, birds fed diets VCPM1 and MCPM1 had reduced weight gain (10-31 d) compared to pelleted diets, due mainly to decreased feed intake. Another possible reason might be that when providing coarser diets, more energy will be utilized for energy-draining grinding function, growth, and maintenance of the gizzard and less energy will be available for body growth, which will contribute to decreased body weight gain (Pacheco et al., 2013; Parsons et al., 2006). This theory is supported by the finding that birds fed heterogenous diets had the highest relative gizzard weight compared to the pelleted-only diets. Also, the higher growth response obtained by pelleting meals may be explained by decreased feeding time and, thus, decreased energy expenditure (Jensen et al., 1962). This theory is supported by the finding of this experiment that birds spend less time eating a pelleted diet compared to heterogeneous diets (Table 14).

No significant difference was observed in feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) of birds fed pelleted-only diets. So increasing the microstructure did not influence broilers' performance. In agreement with the current study, Amerah, Ravindran, et al. (2007b); Engberg et al. (2002) fed broilers a pelleted diet derived from a wheat grain that was either coarsely or mediumly/finely milled and observed no influence on FI, BW, and feed per unit gain. According to Svihus, Kløvstad, et al. (2004), neither the wheat's particle size nor the type of grinder had an impact on the feed intake of broilers-fed pellets. Despite disparities in particle size distribution after pelleting, Péron et al. (2005) showed no influence on the feed intake and body weight of broilers when using finely and coarsely ground wheat in pelleted diets (GMD of 380 and 955 µm, respectively). It is also true that the quality of the pellets has an impact on the performance of the birds. In this study, overall performance was similar among birds fed pelleted diets as the pellet durability was also similar among the pelleted diets which help to maintain similar feed intake and thus feed per gain.

Therefore, it is proposed from this experiment that a much coarser grinding than the normal 3 mm hammer screen can be used without any detrimental effect on birds when fed pelleted diets. With the increased size of the hammer mill screen, there could also be a considerable saving of fossil fuel in the grinding process as expected. However, the performance results regarding the effect of heterogeneous diets were not promising and it is possible to speculate that diet selection might be an issue based on a significant rise in the coefficient of variation for birds fed heterogenous diets.

4.2.2 Gizzard characteristics

The results showed the positive effect of microstructure on gizzard development and functionalities. The relative weight of the gizzard was significantly higher in heterogeneous diets when compared to the standard pelleted diet as expected. The diet composition was different in this experiment compared to the other previous studies but similar results were obtained for the relative gizzard weight and contents. Preserved coarse particles in heterogeneous diets exert stimulatory grinding effects thus increasing the gizzard weight. Due to the stimulative effect of the increased grinding activity on the size of the two pairs of gizzard muscles, the enlarged size of the gizzard is a natural result of an increased necessity for particle size reduction (Duke, 1992). When structural elements like hulls, wood shavings, and whole or coarsely crushed cereals are introduced to the diet, it has been reported that the gizzard's volume may significantly increase, sometimes more than doubling its original size (Svihus, 2011). Additionally, studies have indicated that when whole grains or insoluble fiber are included in the diet, the volume of the gizzard increases significantly (Amerah & Ravindran, 2008; Hetland et al., 2003). After giving quails a high-fiber diet for 14 days, researchers noticed a similarly rapid increase in the size of the gizzard with contents and a similarly rapid decrease in the size of the gizzard after switching to a low-fiber diet for 14 days (Starck, 1999). The purported advantages of a bigger gizzard include enhanced intestinal motility and improved nutritional digestion due to efficient gizzard grinding (Amerah, Ravindran, et al., 2007a), however, no improvement in the digestibility of nutrients was observed in this experiment.

The present data shows that the relative gizzard weight was similar in birds fed pelleted diets (SP, VCP, and MCP). This might be due to the presence of a similar microstructure between the pelleted-only diets after pelleting which exerts a similar response to the gizzard weight. This implies that pelleting lowered the proportion of coarse particles which is in accordance with the results of the comparison of particle size in the mash and pelleted diets obtained in this experiment (Figure 25). According to Svihus, Kløvstad, et al. (2004), the narrow space between the pellet rolls and the pellet die during pelleting makes large particles particularly vulnerable to grinding, which could account for why the procedure tends even out variances in particle distribution. Pelleting decreased the proportion of coarse structure, and the function of the gizzard’s need for grinding has interfered which results in similar gizzard weight in pelleted diets. When compared to mash feeding, birds fed pelleted diets had reduced weights of the gizzard and intestinal tract (Engberg et al., 2002; Munt et al., 1995; Nir, Twina, et al., 1994). According to Svihus (2011), the presence of structural components is the primary factor that simulates the effects of particle size on gizzard growth. So the diets with coarser microstructure had well-developed gizzard compared to the pelleted diets. Due to its ability to reduce particle size, the pelleting process may have a deleterious impact on gizzard development. This demonstrates that the poultry diet has to contain coarse particles for effective gizzard development and that these particles can be preserved by avoiding the pelleting process.

This experiment also shows that the relative contents weight was also significantly lower in birds fed a standard pelleted diet than in those fed heterogeneous diets and no difference was observed in only pelleted diets (SP, VCP, and MCP). Amerah, Ravindran, et al. (2007b); Nir et al. (1995) found that birds fed pelleted feeds had lower relative gizzard weight and content than those in mash feeds. These results may suggest that pelleting decreased the grinding requirements of the gizzard so that its function was reduced to that of a transit organ. The coarser microstructure present in the heterogeneous diets is retained longer in the gizzard for proper grinding, so the volume increases substantially. Accordingly, Hetland et al. (2003), observed increased contents volume when diets with whole cereals of insoluble fibers are fed.

It is clear that birds fed heterogeneous diets and pelleted diets have a tendency for comparable gizzard digesta pH as no significant differences were observed between the dietary treatments. In addition to the stimulatory effect of gizzard activity on acid secretion, dietary structure, such as coarse texture in the diets, results in an increased gizzard volume and longer retention period, allowing for higher hydrochloric acid (HCL) production and pH decrease. As discussed in the literature review, the pH of the gizzard content decreases when structural components such as whole or coarsely ground cereals or fiber materials like hulls or wood shavings are added (Engberg et al., 2004; Gabriel et al., 2003; Huang et al., 2006). However, for pelleted diets, due to the lack of structural components broilers consumed litter which acts as a structural fiber and increases the gizzard activity and thus the digesta retention time. This increases the HCL secretion and reduces the pH. This indicates that birds fed both pelleted diets (due to high litter consumption) and heterogenous diets (due to more coarse cereals) have a similar effect on pH regulation.

In the current trial, no difference in nutrient digestibility, either starch or protein, was found. This indicates that birds can digest both heterogeneous diets and pelleted diets equally effectively. However, Svihus, Juvik, et al. (2004) observed increased starch digestibility by the replacement of ground wheat with whole wheat prior to pelleting, and Hetland et al. (2002) also reported increased starch digestibility by post-pelleting replacement of ground wheat or barley with whole wheat or barley. In heterogeneous diets, the coarse structure promotes gizzard activity, lengthens gizzard retention time, and may reduce the transit rate of digesta through the gizzard, increasing the period that nutrients are exposed to digestive enzymes and potentially enhancing nutritional digestibility. Synchronized with digestion and absorption in the small intestine, the gizzard releases its contents. When food passes through the proventriculus and gizzard, the gizzard-mediated vasovagal reflexes in addition to the production of cholecystokinin (Svihus, Juvik, et al., 2004) in the pyloric region stimulate pancreatic secretions such as amylase, trypsin, etc. which aid in starch and protein digestion. According to Gabriel et al. (2003), a lower pH of the gizzard contents may boost pepsin activity and enhance protein digestion.

So the gizzard pH and digestibility data can be summarised as follows. It shows that the volume of gizzard content and retention time increase when fed heterogeneous diets, resulting in longer contact with the secretion of digestive juices (HCL) which lowers the pH, and pepsin which facilitates protein digestion. In addition, the well-developed gizzard also efficiently grinds the microstructure, passing the finer particles into the duodenum where, due to their increased surface area, they come into contact with pancreatic enzymes enhancing the digestibility of starch and proteins.

The gizzard contents' particle size distribution revealed that the birds fed MCPM1 retained more of the particles (> 1.6 mm). As MCPM1 is a heterogenous diet with a mixture of pellets and coarse structure (mostly > 1 mm), and birds fed MCPM1 diet had relatively larger gizzard weight and content weight it is obvious to expect more coarse particles in the gizzard than only pelleted SP and MCP diets. As there was no significant difference in particles greater than 2.8 mm when comparing SP with other diets, this could be due to the presence of high litter in SP-fed birds. While comparing the particle size of the gizzard with the particle size of the feed, the proportion of particles > 2.8 mm was much higher in the gizzard contents. This reflects that birds consumed litter when structural components are lacking in the diet.

When compared to SP-fed birds, the percentage of the litter retained in MCPM1-fed birds was approximately half as high, although there was no significant difference between the diets. MCPM1 is a diet with a more coarse structure present on it compared to the finely ground and pelleted SP diet. It has been demonstrated that chickens consume a large amount of litter material (Santos et al., 2008) and that the amount consumed is influenced by the texture of the litter material (Malone et al., 1983). Similar findings were made by Hetland et al. (2005) and Hetland and Svihus (2007), however, they also demonstrated that ingestion of litter material increased with decreasing diet coarseness. In our study, the difference in the dietary microstructure between these observations indicates that the birds eat more litter in birds fed SP diet. In absence of coarse structure in the diet, birds ate litter, indicating that birds may eat litter to compensate for the lack of structural components in the feed. Wood shavings were used as a litter in this experiment which may be an alternative source for structural components in diets with a low microstructure that acts as a gizzard stimulating agent.

Even though no differences in nutrient digestibility or particle size distribution in the various intestinal segments were seen, the microstructure of the diets had a favorable impact on gizzard weight. Coarse microstructure not only makes the gizzard size bigger but also significantly boosts its capacity to store feed. In addition, it has also been demonstrated that broilers consume a lot of coarse fiber-containing litter material, a potent gizzard stimulant when structural components like coarse cereals are missing in their diets, standard pelleted diet. This helps to balance the nutrient digestibility and particle size with birds fed heterogenous diets. It seems clear that birds can tolerate coarse microstructures without experiencing any harmful consequences, allowing for coarser grinding to be used in the preparation of broiler feed, which will also assist to lower production costs.

4.2.3 Feeding behavior

Despite a numerical increase in eating time with increased coarseness, no significant differences were found in eating time. It can be speculated that when the diet contains particles with similar structures, birds don't have to spend more time finding the big particles, but when the diet has particles of varied structures, they search for big particles. According to observations carried out by Jensen et al. (1962) of the eating habits of birds given both pelleted and mash diets, mash-fed chicks spent 18.8% of a 12-hour day on eating, whereas pellet-fed chicks ate only 2.2 percent of the time. As mentioned by Nir, Hillel, et al. (1994), the uniformity of feed particles is thought to be significant for good performance for broiler given mash diet, as the birds spend less time finding and selecting the larger particles. From a very early age, birds can select diets with different sizes of particles (Neves et al., 2014). Neves et al. (2014) in their review paper stated that the dimension of the beak determines the selection of feed particles, so big particles in diet are of great importance with the increase in age. Due to the similar structure birds spent less time eating when offered pelleted diets and more time when the birds were raised in heterogeneous diets that were not uniform in size.

Also, the result of particle size preference of two heterogeneous diets, VCPM1 and MCPM2, showed that the feed particles > 2.8 mm decrease over time. In this case, both diets were heterogeneous and the size of pellets in the heterogeneous mixture was around 3 mm in diameter. It makes sense that when consuming a varied diet, birds would have more options for choosing large particles, mostly pellets. Therefore, it was evident that pellets and larger particles (>2.8 mm) had disappeared from both diets, and birds displayed a pronounced aversion to smaller particles and/or fines, supporting the earlier data (Portella et al., 1988; Schiffman, 1969). This showed that the particle size of the coarse cereal particles in the heterogenous diets is still smaller for birds to pick up as Portella et al. (1988) proposed particles for birds greater than 2360 μm for birds after 17 days old. From an early age, birds can choose between different-sized feed particles, and as they get older, they tend to choose larger particles (Nir, Shefet, et al., 1994; Schiffman, 1968).

It is clear from these two feeding trials that broilers favor large pellets over the coarse structures included in the diets. In addition, birds who were fed pelleted diets spent less time eating than those that were offered heterogeneous diets thus increasing their productive energy.

4.2.4 Particle size distribution in small intestine and excreta

Even though the moderately coarsely and very coarsely pelleted diets had coarser structure than the normal standard pelleted diet, mastersizer analysis of digesta particle size in terms of D10, D50, and D90 showed no significant difference in all segments of the small intestine. The proportion of large particles increases when the intestinal contents moved from the upper to the lower part of the small intestine. This shows that the small particles increasingly disappeared while the fraction of big particles to a large extent remains intact. It is clear that broiler chickens can grind coarse particles efficiently because of improved gizzard function when coarse structures are present in the diets. This is consistent with the findings of Svihus, Herstad, et al. (1997); Svihus, Newman, et al. (1997) who did not find any variations in digesta particle size with whole or ground barley in the diet. Nor did Amerah, Ravindran, et al. (2007b) find any difference between coarse and fine particles grinding in the gizzard. In addition, when fed to geese, Moore (1999) found no difference in the digesta particle size when comparing coarsely cut grass with finely cut grass. It was discovered that the size of the particles emerging from the gizzard was, in contrast, incredibly stable. This is consistent with the idea that before exiting the gizzard, the particles are reduced to a specific size. In comparison to diet SP, coarsely ground pelleted diets (VCP and MCP) resulted in an increase ($P < 0.05$) in the amount of particles in the duodenum that was between 0.1 and 0.2 mm (100 µm to 200 µm) in size. This demonstrates that diets that are coarsely ground and pelleted are ground in the gizzard more effectively than regular standard pellets, to a size between 0.1 and 0.2 mm. According to the current study, more than 50% of the particles in the duodenum are smaller than 0.1 to 0.2 mm, while 50 % of the particles in the jejunum and ileum are below 0.2 to 0.5 mm (200 µm to 500 µm) when measured in volume percent (Table 14, 15, and 16, respectively). This demonstrates that the gizzard is capable of reducing food particles to less than 0.2 mm in size before they enter the duodenum and that the proportion of coarse particles used in this experiment does not exceed the capacity of the gizzard to grind finely.

Overall, it can be seen that, regardless of the feed's initial particle size, the gizzard has the extraordinary capacity to grind feed components to a remarkably constant range of particle sizes. Moreover, the majority of particles in the duodenum ranged in size from 16 µm to 751 µm (Table 13), while Ferrando et al. (1987) found that the threshold particle size is 500 to 1,500 µm before leaving the gizzard. Hetland et al. (2002), found that many particles were smaller than 100 µm, with as many as 50% of the particles in the duodenum being less than 40 µm. This disparity in particle size between the results of the current study and those of Hetland et al. (2002) may be the result of the different methodologies used in both studies, which only included particles up to 800 µm in size in their study. Additionally, there was no discernible difference in the size of the jejunal particles among three pelleted diets with different coarseness, which is consistent with the observation of Xu, Stark, Ferket, Williams, Pacheco, et al. (2015) that the digesta particles in the jejunum displayed a comparable distribution with 0, 25, and 50% inclusion of diets containing coarsely crushed corn (CC).

The particle size determination of excreta revealed that both pelleted (VCP) and heterogeneous diets (VCPM2) were effectively ground in the gizzard of broiler chicken. Approximately 10 % of the excreta particles were greater than 1 mm (1,000 µm) which is in general agreement with the previous finding of Svihus, Herstad, et al. (1997) who discovered a similar 10 % of digest particles greater than 1 mm approximately when fed either whole or ground barley. Likewise, when 16-day-old broilers were fed a diet containing 700 g/kg of whole barley, Svihus, Newman, et al. (1997) observed very few whole barley kernels in their droppings. Therefore, as mentioned previously, the gizzard is particularly effective at processing whole or coarsely ground cereals.

From the above discussion, it can be concluded that the gizzard has a remarkable ability to grind the coarser particles of the diets to a critical size where 50% of the particles are below 200 µm before entering the duodenum.

5. Conclusion

It can be concluded that increasing the diameter of the screen hole increases the coarseness of the ground cereals which can increase the grinding capacity and reduces energy consumption. Pelleting tends to reduce the relative proportion of coarser particles > 1 mm so preservation of coarse microstructure is of great importance in the broiler feed. Broiler performance was higher in pelleted-only diets compared to heterogeneous diets due to higher feed intake. Although the feed intake and body weight gain were not improved by using the heterogeneous diets, positive effects of coarse cereals can be seen on gizzard development which indicates that diet selection is a limiting factor in this experiment. Nutrient digestibility and particle size distribution of the intestinal contents were not affected which showed that birds can handle the coarse structure through effective gizzard stimulation. It seems that both the micro- and macrostructure can be increased for commercial broiler feed production. Therefore, for cost-effective feed production and at the same time improved broiler health and performance, coarser grindings would be advantageous. More experiments are needed in the future to study the interaction of macro and microstructure in broiler diets.

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