The effect of maize and soybean oil cake particle size on broiler growth performance and gastrointestinal development

by

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Declaration

I, Chrisjan Bakker, declare that this dissertation, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution.

CJ Bakker
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List of abbreviations

BW	Bodyweight
F	Finisher
FCR	Cumulative feed conversion ratio
FI	Cumulative feed intake
G	Grower
GLM	General linear model
H0	Null hypothesis
HA	Alternate hypothesis
PEF	European performance efficiency factor
PF	Post-finisher
PS	Pre-starter
S	Starter
WI	Water intake
AGP	Antibiotic growth promoter
GIT	Gastrointestinal tract
GMD	Geometric mean diameter
GSD	Geometric standard deviation
AME	Apparent metabolisable energy
PDI	Pellet durability index
CP	Crude protein
AA	Amino acids

T	Treatment
RCBD	Randomised complete block design
ANOVA	Analysis of variance
LSD	Least significant difference
CRD	Complete randomised design

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Abstract

Globally the demand for food, especially for food of animal origin, will double by the year 2025 and almost tripled by the year 2050. This means that animal nutritionists need to explore ways of ensuring that there will be adequate amounts of animal protein available for consumption by using natural resources more efficiently. Improving the efficiency of utilisation of modern day broiler feeds may be key to achieve high levels of animal protein production at affordable cost. The manipulation of the particle size of the two main ingredients of broiler feed namely, maize and soybean meal, may improve efficiency of overall broiler performance.

This trial assessed the effect of maize and soybean particle size on broiler performance, gizzard and proventriculus development and pellet hardness. Additionally, different combinations of maize and soybean meal particle sizes were compared with the aim of finding the optimal combination where commercial value can be maximised. This trial had 12 treatments with 10 replications for each treatment. The twelve treatments contained different combinations of maize and soybean meal particle sizes. Seven thousand two hundred day-old chicks were placed in a broiler facility at 23 birds/m^2 and raised up to slaughter at 35 days-of-age on a three-phase feeding program. Bodyweight (BW), feed intake (FI), feed conversion ratio (FCR), production efficiency factor (PEF) and mortality were recorded on a weekly basis. Gizzard and proventriculus measurements were taken on 7, 14 and 35 days-of-age and pellet hardness were measured for the grower and finisher phase diets.

The production efficiency factor (PEF) values increased as the particle size of the soybean meal in the diet was reduced, with treatment 4 (15% coarse maize; 2 mm soybean meal) giving the highest value. When the diet contained soybean meal milled on a roller mill with a screen size of 4 mm (treatment 3 and 5), broilers that received treatment 5 containing 30% coarse maize outperformed treatment 3 containing only 15% coarse maize in terms of BW and FCR. Maize and soybean meal particle size in a pelleted diet did not have a significant effect on gizzard development during the first two weeks, but it had a significant effect on the gizzard development during the latter stage of production. Furthermore, no effect was seen on the proventriculus development. Pellet hardness and pellet durability were influenced by the maize and soybean meal particle size with treatment 4 that contained 2 mm soybean meal and 15% coarse maize resulted in the hardest pellets.

Up to now, soybean meal was mostly used by feed mills as received from the suppliers without any further processing. The recommendation from this study is that the soybean meal destined for broiler diets should rather be milled through a sieve of 2 mm screen size. The smaller soybean particle size promotes higher bodyweights and better FCR and PEF values. The improved feed efficiency obtained with the smaller particle sizes means that less resources can be used to produce food for humans. For optimum broiler performance a pelleted diet with a 15% coarse maize and 2 mm soybean meal combination must be used.

Chapter 1

Introduction

According to predictions, during the next 25 to 30 years the global population will increase from 6 to 8 billion people and can possibly even reach 10 billion people in 2050 (Flachowsky *et al.,* 2005). The pressures due to a rapidly growing world population will worsen the already existing problems of food insecurity and nutrient deficiencies. Ever since plants were domesticated, people tried to change the characteristics of the wild varieties to better utilise the available natural resources. Plant breeders achieved great progress which made the huge increase of world population, seen in the last few decades, possible. The supply of food, especially food of animal origin, will need to increase by 100% by the year 2025 (McCalla, 1999) and almost 200% by the year 2050 (Vasil, 1998). This massive growth of population and demand for animal derived protein is mainly due to a change in the eating patterns of people in developing countries, as a consequence of better economies and improved lifestyles. The ever-increasing pressure on the natural resources, will pressurize using resources more efficiently to produce food. This will especially have huge implications on the production of raw materials used as feedstuffs, because almost 60% of the plant mass produced by farming enterprises worldwide is used in animal nutrition and the conversion of plant materials as feeds into foods of animal origin is associated with considerable losses in energy, protein and various other nutrients (Flachowsky, 2002).

Animal nutritionists need to explore ways of ensuring that there will be adequate amounts of animal protein available for consumption by using natural resources more efficiently. One of the first steps in the manufacturing process of commercial poultry feed entails the mixing of ground seeds such as maize and wheat with protein meals such as soybean meal and by product meal. Up to date the feed manufacturing industry had limited control over the particle size of especially protein meals, as most of them are supplied and delivered after being ground by the processors. On the other hand, the particle size of seeds, like feed cereals and legumes, may vary at the feed mill according to the processing by the feed plant itself. A reduction in the size of the particles involves a two-step process which entails the disruption of the outer seed coat and the exposure of endosperm. Ongoing reduction in particle size will increase both the number of particles and the surface area per unit volume allowing greater access to the enzymes inside the digestive tract (Goodband *et al.*, 2002). Furthermore, the reduction in particle size will also increase the ease of handling as well as improved mixing of the feed ingredients (Koch, 1996). Unfortunately, there are practical limitations in particle size reduction, as the chickens may experience difficulties in ingesting very coarse or very fine particles (Koch, 1996). Lately, feed particle size came under the spotlight, as a result of the feed industry searching for ways of improving feed utilisation and enhance production efficiency. Guidance as to what the best particle size is, however, have been contrasting, as the results from animal feeding trials are influenced by a number of factors including feed physical form, diet constituents, grain ingredient type, endosperm hardness, grinding method, pellet quality and particle size distribution (Amerah *et al.,* 2007a). The extent of particle size reduction of these seeds may have a dramatic influence on various facets of poultry production like overall bird performance and digestive tract development (Amerah *et al.*, 2007a).

The anticipated banning of antibiotic growth promoters (AGPs) in animal feeds has inspired nutritionists to take a look at unconventional feed management action plans to improve the health and digestive efficiency of broiler chickens. Enhancing of the development of the gizzard is one of these nutritional strategies, which can be realised by changing the feed particle size (Nir *et al*., 1995). A well-developed gizzard is correlated with enhanced gut motility (Ferket, 2000) and can possibly avert pathogenic bacteria from getting into the small intestine (Bjerrum *et al.,* 2005), which will then dramatically reduce the risk of coccidiosis and other enteric diseases (Cumming, 1994). Also, Lentle (2005) speculated that when poultry are fed a greater proportion of coarse particles in their diet it will result in more coarse particles moving through the gizzard, which can improve the permeability of digesta to enzymes and improve digestive efficiency.

Inclusion of a coarser grain reduces energy expenditure during feed manufacturing with the grinding process of raw materials for broiler feeds accounting for one of the highest energy expenditures in a feed mill (Reece *et al*., 1985). A bigger screen size in the hammer mill (from 4.76 to 6.35 mm) has shown to lower the total energy consumption of the mill by 27% (Reece *et al*., 1986). However, coarse particles are said to have a negative influence on pellet quality (Abdollahi *et al.,* 2012), but there is no concrete evidence for this claim. In every trial asessing the effects of feed particle size in broiler diets, it is very important that the physical form of the diet (mash vs. pellets) is also taken into account. The findings of various trials show that the diets containing large-grain particle size, had a bigger effect on broiler performance when birds were fed mash diets vs birds fed pelleted or crumbled diets (Nir & Ptichi, 2001).

Improving the efficiency of utilisation of modern day broiler feeds may be key to achieve high levels of production efficiency without using AGPs. The manipulation of the particle size of the two main ingredients of broiler feed namely maize and soybean meal may increase the nutrient utilisation of broilers thus resulting in better performance (Siegert *et al*., 2017), while at the same time improving pellet durability (Briggs *et al.*, 1999). Grinding these ingredients will increase the surface area of the feed in the gastro-intestinal tract (GIT) of the broilers thus presenting more nutrients for uptake (Siegert *et al.*, 2017), but the exact effects on the overall performance of broilers, gizzard and proventriculus development and pellet hardness needs clarification.

Aim and objectives

The aim of this trial was to find the optimal particle sizes for both the maize and soybean meal in South African broiler feed. The objectives were to assess various combinations of maize and soybean meal particle sizes on broiler performance, gizzard and proventriculus development and pellet hardness.

Hypothesis of the study

The first null hypothesis (H_0) of this study is that decreasing the particle size of maize and/or soybean meal will have no effect on performance in Ross 308 broilers.

The first alternative hypothesis (H_A) is that decreasing the particle size of maize and/or soybean meal will improve performance in Ross 308 broilers.

The second null hypothesis (H_0) of this study is that maize and/or soybean meal particle size will have no on gizzard and proventriculus development in Ross 308 broilers.

The second alternative hypothesis (H_A) is that maize and soybean meal particle size will have a significant effect on gizzard and proventriculus development in Ross 308 broilers.

The third null hypothesis (H_0) of this study is that decreasing maize and soybean meal particle size will have no effect on pellet durability.

The third alternative hypothesis (H_A) is that decreasing maize and soybean meal particle size will have a positive effect on pellet durability.

Chapter 2

Literature Review

2.1. Introduction

Very few studies have been conducted with regards to the optimum particle size of different feed ingredients for optimum poultry production. Currently used highly processed, pelleted diets conceals the effect of particle size, but a few studies have shown that the effects of feed particle size on poultry performance may be supported even after pelleting (Kilburn & Edwards, 2001).

There seems to be some common ground that for broiler diets based on maize or sorghum, optimum particle size is between 600 and 900 μm (Cabrera, 1994). Current accessible data sets agree that grain particle size is more important in mash diets than in pelleted or crumble diets (Calet, 1965). Even though it has been hypothesized that finer particles increase substrate availability for enzymatic digestion, some studies have shown that more uniform coarser particles improve the overall bird performance when maintained on mash diets (Siegert *et al*., 2018). This contrasting outcome may be because of a better developed gizzard due to the coarser particles in the diet (Jacobs *et al*., 2010). A better developed gizzard is correlated with more grinding activity, giving rise to increased gut motility and better nutrient digestibility (Jacobs *et al*., 2010).

Having a fine particle size is said to produce pellets of high quality (Martin, 1985), but it significantly increases the amount of energy used during milling (Dozier, 2002). Methodical investigations on the link between feed particle size and diet uniformity with overall bird performance, gut health and pellet quality are justified when efficiency is to be maximised in respect of the energy expenditure of grinding. The following literature review focusses on feed ingredient particle size, gizzard and proventriculus development, pellet hardness and the overall effect of broiler performance.

2.2. Processing of feed for broilers

Broiler feed consist of various raw materials that needs to undergo numerous processes before it can be fed to broilers. These different processes can also play a vital role in how animal nutritionists formulate and define broiler feed.

2.2.1 Methods of grinding

In modern day feed mills particle size reduction of grains and other feedstuffs is mostly achieved by two processing methods namely: the hammer mill and the roller mill (Koch, 1996).

Inside the hammer mill there is a set of hammers moving at very high speed in a hollow grinding chamber, which beats the grains until the particles are small enough and able to pass through a screen of a specific size. The particle size generated, ultimately depends on the size of the openings on the screen and the speed at which the hammers rotate (Koch, 1996). The efficiency of the hammer milling process will be influenced by numerous factors, including grain type, grain moisture content, screen size, screen area, peripheral speed, hammer width and design, number of hammers, hammer tip to screen clearance, feed rate, power of the motor and speed of air flow through the mill (Martin, 1985).

The roller mill consists of one or various pairs of horizontal rollers in a frame that is able to support their weight. The distance between these rollers can be different, depending on the particle size required. The grains are crushed by a constant compression force as they move through the rotating rollers, resulting in a smaller particle size. Modern day roller mills are more cost effective and will result in a lower energy expenditure for the grinding process (Dozier, 2002). Roller mills achieve a more uniform particle size and the ground grains will hold a lot less fines. One disadvantage of the roller mill is that grains that are smaller than normal can pass through the grinding process in the roller mill (Douglas *et al*., 1990) and the shape of the resulting particles is more uneven, being cubic or rectangular (Koch, 1996) as opposed to those from a hammer mill, which will normally be spherical with more a more even shape (Reece *et al.,* 1985). Most commercial feed mills still use the hammer mill for grinding grains due to its lower maintenance and ease of understanding and usage.

The grain type will ultimately have a huge influence on the particle size. Various studies have shown that grinding different grains with the same mill under similar conditions would result in ground grains with varying particle sizes (Lentle *et al.,* 2006). This means that when grinding different grains, separate screen sizes may have to be used depending on the type of grain in order to acquire the desired particle size distribution. It is important consider that not all batches of the same grain type, ground by the same mill type in similar conditions will result in the same particle sizes. Different batches of grain can have variations in endosperm hardness thus resulting in different particle sizes. Lentle *et al.* (2006) showed that grains from three different cultivars of wheat ground in a hammer mill using the same sieve resulted in three different particle size distributions.

Whether the feed ingredients were hammer or roller milled had no significant effect on the overall broiler performance when the birds were fed diets which had the same geometric mean particle diameter (Nir *et al.,* 1990).

2.2.2. Measuring particle size

Particle size can be described as the average diameter of the individual particles of feed or simply the "fineness of grind" of the feed. Earlier scientists (Eley & Bell, 1948) only used common terms like: fine, medium and coarse when describing the size distribution of particles, but these terms did not allow for accurate and helpful comparisons between datasets. In order to overcome this problem, methods were then developed to quantify particle sizes distribution more accurately. The average particle size is specified as the geometric mean diameter (GMD), expressed in mm or microns (µm) and the scope of variation is defined by the geometric standard deviation (GSD), with a bigger GSD showing lower uniformity.

Geometric mean diameter and geometric standard deviation only describes the particle size distribution accurately, expressed as log data, when it is distributed parametrically, i.e. log normally (Lucas, 2004). Feed particle size distribution is normally obtained by sieving of a specific pre-set weight that compounds a representative sample (Baker & Herrman, 2002). This representative sample is then passed through a specific sieve stack on a shaker or by hand for approximately 10 minutes. The mass of particles retained on each screen size is then recorded, and the GMD and GSD of the sample calculated using a standard formula or computer software. Another way to determine particle size distribution is by wet sieving, but this gets applied on digesta and excreta samples most of the time. In the wet sieving method, feed samples get suspended in 50 mL of water and left to sit for 30 min before sieving to certify sufficient hydration (Lentle *et al.*, 2006). The sample is then washed through a set of sieves and the material retained on each sieve, plus a representative sample of elute, is filtered afterwards and dried for 24h at 80°C. The mass of the retained particles from each sieve then gets expressed as a percentage of the total dry matter recovered.

2.2.3 Feed form

There is a lot of studies showing the interaction between particle size distribution and the physical form of feed in broiler diets for weight gain and feed intake. Results obtained from various trials propose that grain particle size is more important in mash diets than in pelleted or crumbled diets (Reece *et al.,* 1985). Feeding broilers a pelleted diet is known to enhance weight gain, feed intake and feed efficiency regardless of the grain source (Calet, 1965). This development has been assigned to higher density, better starch digestibility as a result of chemical changes during the pelleting process, increased nutrient intake, reduced feed wastage and less energy spent on the consumption of feed (Calet, 1965). In laying hens, the same improvements were seen (Morgan & Heywang, 1941). However, Hamilton & Proudfoot (1995) suggested that laying hens on a mash diet accomplished better production performance than those fed a crumbled diet.

2.2.4. Pelleting of feed (including pellet quality and durability)

Good quality feed is the greatest expense in modern day broiler production systems constituting as much as 60–70% of the total production cost. The various feed ingredients are the major contributor to this high cost of feed. Various processing methods also contributes to the cost of feed (Nolan *et al.,* 2010). However, these processing methods can be valuable tools to improve broiler production efficiency. This means that methods of processing feed prior to ingestion is attracting more and more attention of the leading poultry nutritionists worldwide as they strive to improve the value that feed is adding to the production chain. Different action plans can be implemented to further improve feed processing techniques; but, the expense of each action plan must be thoroughly balanced against the realisable performance advancements and detrimental effects in the target animal (Behnke, 1996). In poultry feed production, pelleting is the most common processing strategy with the aim of better performance. Smaller feed particles are bound together during pelleting through very high mechanical pressure, moisture (steam) and high heat. The mash needs to be treated prior to pelleting (Skoch *et al.*, 1981), this usually entails the addition of steam to the mash feed. Feeding pellets to poultry improves the production by improving the feed intake (FI), growth and feed efficiency. In contrast, the pelleting process may also have a negative effect on the production performance of the birds because of the physical and chemical changes that is a result of this process (Svihus, 2011).

Pellet quality can be defined as the potential of the pellets to resist disintegration and corrosion during mechanical handling (bagging, storage and transport) without fragmenting and to get to the feeders intact, with a low percentage of fines (Amerah *et al.,* 2007a). A positive correlation exists between feed efficiency and pellet durability (Carre *et al.,* 2005). Disintegration and corrosion are the two main causes of pellet attrition. Disintegration occurs when the pellets break up into smaller particles and fines, while corrosion can be described as the breaking up of the surface area of the pellets, resulting in an uneven surface (Thomas & Van der Poel, 1996).

The pellet durability index (PDI) can be used to describe pellet quality (PDI) (ASAE, 1987). The Pfost tumbling can device enables feed mills to give an accurate estimate of the pellet durability. The procedure of Pfost (1963), states that pellet durability is attained by causing fines through a grinding action of pellets moving across each other and against the wall of the can. To test the pellet durability, the pellets are passed through a sieve to get rid of all the fines. The sample is then tumbled in the Pfost device for a pre-set time period (Thomas & Van der Poel, 1996). After this specific time period, the pellet sample is sieved again to remove all the fines post tumbling. The pellet durability index is then calculated as the ratio of intact pellets after tumbling to pellets at the start.

Pellet hardness can also be indicative of pellet quality, but is seldomly considered. The pellet harness test may be important because it can determine whether the pellets are hard enough to avoid breaking under the pressure in bulk bins. Pellet hardness can be measured through equipment that is able to determine the amount of force needed to break or crush a pellet. The ‘Kahl’ device is one such device. When using the Kahl device, a pellet is placed between two posts. The static pressure applied on the pellet is then slowly increased until the pellet breaks, upon which a measurement of the amount of pressure applied is recorded (Thomas & Van der Poel, 1996). Other devices that can be used for pellet hardness measurements includes the Schleuniger test apparatus, Pendulum test device, Instron device, Kramer shear press (Thomas & Van der Poel, 1996) and Texture Analyser (Svihus *et al.,* 2004).

It is important to consider that even though there are general advancements in PDI and pellet hardness due to processing methods during pelleting process, like higher priming temperatures (Abdollahi *et al.*, 2012), addition of generally available binders and/or moisture (Abdollahi *et al.*, 2012) and bigger pellet size (Abdollahi *et al.*, 2012) have improved both measurements, a thorough analysis of these data sets shows that in most scientific trials the level of advancement in pellet hardness was bigger than in the PDI. This may lead to the conclusion that the effect of various processing methods is more pronounced on disintegration than on corrosion resistance. The positive effects of these processing methods on pellet hardness, as an indicator of pellet quality, are not seen or noticed when only the PDI is evaluated. Parsons *et al*. (2006) concluded that samples with different pellet hardness (1662 g of force for softer pellets and 1856 g of force for harder pellets) can have almost equal pellet durability's. It is therefore important to measure the pellet hardness and pellet durability when trying to evaluate the overall pellet quality.

2.3. Effect of the particle size on feed manufacturing

The particle size of feed ingredients will have a major influence on the feed manufacturing processes and the cost thereof. Furthermore, the particle size of the feed constituents will ultimately dictate the pellet quality and feed efficiency.

2.3.1. Particle size and the cost of feed

In poultry production systems, feed is the single biggest expense. Pelleting constitutes the biggest energy cost to a feed mill, with ingredient particle size reduction being the second biggest for broilers (Reece *et al.,* 1985) and the biggest energy expense in the feed manufacturing industry for laying hens, as this feed is usually not pelleted (Deaton *et al.,* 1989). Feed particle size reduction lowers the overall production rate of a feed mill and has a higher energy cost. If this high energy usage at the feed mill to reduce the particle size of the feed ingredients can be avoided, it could mean significant feed price drops would be possible.

Reece *et al.* (1986) concluded energy savings of up to 27% was possible when the hammer mill screen size was increased by 1.60 mm. Unfortunately, there is no linear relationship between energy input and hammer mill screen size. The amount of energy needed to mill maize from a GMD of 600 µm to 400 µm is 100% more than the energy needed to decrease the particle size from 1,000 to 600 µm (Wondra *et al.,* 1995). The effect of particle size reduction on production rates are also non-linear (Wondra *et al.,* 1995). Various trials have shown that fineness of grind of grain has no effect on the pelleting rate in a feed mill (Martin, 1985) or the amount of energy needed for pelleting (Martin, 1985; Svihus *et al.*, 2004). Consequently, any particle size reduction that brings about higher/better production performance in poultry must be adequate to counterbalance the higher cost associated with the finer grinding of the feed ingredients.

2.3.2. Effect of particle size on pellet quality

It is common belief that particle size is inversely related to pellet durability (Angulo *et al.*, 1996) due to the fact that smaller particles have a larger surface area per unit volume. This means that there are more points of contact between these particles which forms a stronger bond (Behnke, 1996). Unfortunately, there is almost no scientific confirmation of this with various studies concluding that the particle size of grain has no influence on pellet durability (Reece *et al.*, 1986; Koch, 1996). Furthermore, contrasting trial results have been acquired from the few studies that correlates particle size with pellet durability (Table 2.1). Carre *et al.* (2005) concluded that wheat hardness had a positive correlation with pellet durability. This study affirmed that the hardness effect was independent of the wheat particle size. Numerous factors such as feed content (dietary protein and oil) (Briggs *et al.*, 1999), mash priming prior to pelleting, pelleting die specifications, and cooling and drying post pelleting will influence pellet durability (Behnke, 1996). Thus, making it difficult to quantify the effect of particle size on pellet durability as various milling parameters may influence the outcome of the trial. Low starch gelatinisation may be the reason for poor quality pellets when the ingredients are coarse (Svihus *et al.*, 2004).

Table 2.1 Effect of particle size on pellet durability (Amerah *et al.*, 2007a)

Grain	GMD (µm)	Pellet durability index	Reference
Maize	910	91^a	Reece *et al.* (1986) [1]
	1024	91^a	
Maize	679	91^a	Reece *et al.* (1986) [1]
	987	91.3^a	
	1289	92.5^b	
Wheat	600	88^a	Svihus *et al.* (2004) [1]
	930	81.2^a	
	1700	80.2^a	
Wheat	380	25^a	Peron *et al.* (2005) [2]
	955	25^a	

[1] Pellet durability was measured using Holmen Pellet Tester

[2] Pellet durability was measured using Eurotest rotary mill.

[a,b]Within each reference, values in a column with different superscripts are significantly different ($P < 0.05$)

GMD: Geometric mean diameter

2.4. Effect of particle size on broilers

The particle size of the broiler feed ingredients may have a massive effect on broiler performance as it dictates the availability of nutrients to the bird. It will also effect the development of various organs in the gastrointestinal tract of the broiler, especially where it gets broken down into smaller particles.

2.4.1. Broiler performance

Even though there is some lack of consistency, the majority of the datasets indicate that when considering broiler production performance, that any particle size effect becomes irrelevant when the birds are fed crumbs or pellets (Table 2.2). Nonetheless, there has been increased interest in evaluating the effects of particle size distribution in pelleted feeds. The ingested pellets dissolve in the crop of the bird, meaning that the particle size of the feed ingredients prior to pelleting may still hold some value (Nir *et al*., 1995). Various scientific trials have shown that feed particle size has no significant effect on broiler performance when offered as a pelleted diet. Reece *et al*. (1986a) concluded that different maize particle sizes had no effect on broiler performance when the starter ration was offered in crumbled form. Likewise, Svihus *et al.* (2004) showed that particle size distribution in a pelleted diet had no effect on broiler performance. Peron *et al*. (2005) also concluded that different particle sizes (GMD, 380 and 955 µm) was maintained after pelleting but it had no effect on any of the performance parameters of modern broiler production. In contradiction, various studies have shown significant effects of the particle size distribution in pelleted feeds that indicates variation among and between different grain types. Lentle *et al.* (2006) used three different cultivars of wheat which respectively yielded different particle size distributions after hammer milling on the same mill. They concluded that the pelleted diet with the coarsest particles yielded a significantly better feed efficiency. Various other studies, using maize-based pelleted diets, have found that a finer particle size distribution yielded better broiler performance parameters than a coarse particle size distribution. Lott *et al.* (1992) concluded that broiler chicks raised on pelleted diets made from coarsely ground maize (GMD, 1196 µm) showed a significantly lower weight gain and poor feed efficiency at 21 days-of-age when compared with broilers on pelleted diets that consisted of maize with a smaller particle size (GMD, 679 µm). Likewise, Kilburn & Edwards (2001) concluded that overall broiler performance and true metabolisable energy were better when the birds were raised on pelleted feeds that included fine maize (GMD, 869 µm) compared to a pelleted feed that contained only coarse maize (GMD, 2897 µm).

Interestingly, Reece *et al.* (1986b) compared different maize-based diets and concluded that with a similar feed intake, fine (GMD, 679 µm) and coarse (GMD, 1289 µm) grindings resulted in better bodyweights and improved feed efficiency, relative to the birds that received medium ground maize (GMD, 987 µm). There was no significant difference between the performance of the birds on the fine and coarse maize respectively. Birds that received a pelleted diet in which the maize fraction consisted of 50% coarse and 50% fine maize performed better than the birds that received a pelleted diet in which the maize fraction was medium ground, despite the GMD of both these diets being similar. These contrasting findings may be linked, in part, to differences in the size distribution after the pelleting process. The differences seem to be based on the type of grain and different cultivars. The particle size distribution of grains after milling can be influenced by the hardness of the grain (Carre, 2004). Currently there is no clear scientific evidence that indicates the effect of the hardness of the grain on particle size distribution post pelleting. In trials consisting of wheat-based diets where the particle size differences remained after pelleting were fed to broilers, it was found that the diets with coarser particles had a better feed efficiency (Lentle *et al.,* 2006). However, in trials where the particle size differences didn't remain after pelleting, it was found that there was no effect on broiler production performance (Svihus *et al.*, 2004a).

Various studies have concluded that significant improvements in broiler performance was observed when medium (600-1000 μm) or coarse (>1000 μm) particle size, compared to fine (<600 μm) particle size, was fed to broilers as mash diets (Reece *et al.,* 1985). In contrast, Nir *et al.* (1994) showed that feed conversion ratio (FCR) was significantly better in birds maintained on maize with a fine (<600) particle size distribution compared to maize with a medium particle size distribution in a mash diet. This clearly shows the inconsistency with regards to the ideal particle size distribution and the scope of the effect it has on overall broiler performance. The way the birds respond to the particle size in the diet will depend on the stage of maturity of the GIT and the physiological age of the bird. Nir *et al.* (1994) showed that particle size had no effect on bird performance during the first week, but the production performance was significantly better when the diets consisted of coarse maize particles vs fine or medium particles for the period between 7 and 21 days-of-age. This may be due to the low level of development of the gizzards in such young birds. They don't have the grinding capacity of the older birds. Jacobs *et al.* (2010) also concluded that there was a linear decrease in the bodyweight of broilers on diets with increasing maize particle size in birds for the first week, with significant effects seen when comparing the two largest (1210 and 1387 μm) and two smallest (557 and 858 μm) particle size distributions.

Eating behaviour may also affect broiler performance when the birds are given a mash diet (Amerah *et al.,* 2007a). Clark *et al.* (2009) showed that birds have a fondness for the bigger particles in a mash feed, indicating that birds may only be consuming the larger particles which may result in nutrient imbalances. To decrease the effect of this behaviour, it is advised that the feed is provided in pelleted form so that all the nutrients in the formulation gets consumed. However, there is a lack of consistency when differentiating between pelleted diets which consists of different particle size distributions. This inconsistency is mainly because of varying GMD of particle size in the trials, feed ingredient inclusion levels of the specific particles, breed of birds and physiological age (Calet, 1965; Doglas *et al,* 1990; Briggs *et al.,* 1999; Baker & Herrman, 2002).

Several studies have shown no significant effect on broiler production performance when pelleted diets containing different particles sizes were used, indicating that pelleting may invalidate the different particle size distributions (Svihus *et al.,* 2004; Amerah *et al.,* 2007b). The size of the pellet and the gap between the rollers and pellet die can influence the terminal particle size in the pellets. A bigger pellet and more space between the rollers and the die should decrease the amount of further grinding of the particles during the pelleting process. Pellets will dissolve in the crop of the bird after consumption, so the important influence of particle size on broiler performance should still be present even after the pelleting process (Abdollahi *et al,* 2012). Pacheco *et al.* (2013) showed that a diet containing coarsely ground maize resulted in lower bodyweights during the first week vs feeding a diet containing finely ground maize. This indicates that young broilers cannot effectively utilise coarse particles, even when fed as a pelleted diet, due to their gizzards not being developed enough yet. Likewise, Kheravii *et al.* (2017) concluded that a diet consisting of a bigger particle size distribution resulted in lower bodyweights and feed intake during the first 10 days. Although, the FCR was significantly better between 24 and 35 days-of-age, indicating that broiler performance could be improved by the feeding of diets containing more coarse particles as soon as the gizzard is well developed. The feed efficiency of broilers were also better when fed wheat based diets where the coarser particle size distribution persevered after pelleting (Lentle *et al.,* 2006).

The percentage of coarse or fine particles in the diet influences the effect on broiler performance (Rougière *et al.,* 2009). Xu *et al.* (2017) showed that replacing 20% of fine maize with coarse maize had no significant influence on broiler performance during the first two weeks, however when half of the fine maize was replaced, FCR and bodyweight were consistently better after two weeks of age. Xu *et al.* (2015) concluded that the replacement of half of the fine maize with coarse maize in a pelleted diet improved the FCR from day 0-35 and day 0-49 and improved the bodyweights from 0-35 days-of-age.

Table 2.2 Effect of particle size on the performance of broilers fed with pelleted diets (Amerah *et al.*, 2007b)

Grain	Age (days)	Particle size (μm)	Gain (g/bird)	Feed intake (g/bird)	Feed/gain (g/g)	Reference
Maize	0-46	910	1998 ^{a}	-	1.934 ^{a}	Reece *et al.*
		1024	2001 ^{a}	-	1.931 ^{a}	(1986)
Maize	0-42	679	1820 ^{a}	3447 ^{b}	1.894 ^{a}	Reece *et al.*
		987	1754 ^{b}	3347 ^{a}	1.908 ^{b}	(1986)
		1289	1800 ^{a}	3400 ^{ab}	1.889 ^{a}	
Maize	0-21	679	748 ^{a}	1048 ^{a}	1.40 ^{a}	Lott *et al.*
		1289	729 ^{b}	1032 ^{a}	1.42 ^{b}	(1992)
Maize	0-16	869	386 ^{a}	-	1.35 ^{a}	Kilburn & Edwards
		2897	382 ^{b}	-	1.39 ^{b}	(2001)
Wheat	0-42	Fine	2007 ^{a}	3512 ^{a}	1.78 ^{a}	Engeberg *et al.*
		Coarse	1997 ^{a}	3478 ^{a}	1.78 ^{a}	(2002)
Wheat	11-30	600	1361 ^{a}	2133 ^{a}	1.57 ^{a}	Svihus *et al.*
		1700	1315 ^{a}	2114 ^{a}	1.58 ^{a}	(2004)
Wheat	7-15	300	440 ^{a}	380 ^{a}	1.28 ^{a}	Peron *et al.*
		955	444 ^{a}	375 ^{a}	1.25 ^{a}	(2005)

^{a,b} Within each reference, values in a column with different superscripts are significantly different ($P < 0,05$)

2.4.2. Gizzard and proventriculus development

Feed particle size influences how the gizzard of the broiler develops, especially during the first week of growth. Nir *et al.* (1994) showed better developed gizzards and lower gizzard pH in 7-day old chicks that were fed a crumbled diet containing a medium or coarse particle size distribution when compared with a crumbled diet that consisted of a finer particle size distribution. The gizzard is a muscular organ that grinds the ingested particles into smaller particles and exposes these smaller particles to the various digestive enzymes (Duke, 1986).

The gizzard can apply mechanical pressure of up to 585 kg/cm^{2} (Cabrera, 1994). Fine grinding of the particles by the feed mill can have a detrimental effect on gizzard size and function. When broilers receive a highly processed pelleted diet with a fine particle size distribution, they tend to have underdeveloped gizzards and the proventriculus becomes enlarged (Taylor & Jones, 2004). When this phenomenon happens, the gizzard tends to lose its grinding capacity and acts only as a transit organ (Cummings, 1994). The weights of the gizzard and the small intestine, relative to the bodyweight, (Nir *et al.,* 1995) can decrease when birds are maintained on a pelleted instead of a mash diets. In mash diets, the particle size distribution of the feed is positively related to the gizzard weight (Nir & Ptichi, 2001) relative to the bodyweight of the bird. However, in pelleted diets, this probably depends on the particle size distribution in the crop after ingestion and dissipation. Peron *et al.* (2005) showed that in wheat based diets consisting of extremely hard wheat, varying particle sizes persisted after pelleting with GMD values of 380 and 955 µm, and that the birds fed pellets that contained coarse particles had significantly higher gizzard weights vs birds fed pellets that contained only fine wheat. The hardness of the wheat probably resisted further grinding during the pelleting process. Amerah *et al*. (2018) showed that when comparing birds fed on a pelleted and a mash wheat based diet respectively, there was no significant difference in the overall morphology of the digestive tract (Table 2.3). A big, well-developed gizzard enhances gut motility (Ferket, 2000) by expanding the levels of cholecystokinin release (Svihus *et al.*, 2004a), which stimulates the secretion of pancreatic enzymes and the gastro-duodenal refluxes (Duke, 1992). Coarser particles in the digesta can cause a slower passage rate through the gizzard (Nir *et al.,* 1994b), exposing the nutrients to the digestive enzymes for a longer period, which can subsequently enhance energy utilisation and overall nutrient digestibility (Carre, 2000).

Protein digestion may be better when the gizzard has a lower pH because of improved pepsin activity (Gabriel *et al.,* 2003). Furthermore, a lower gizzard pH can lower the coccidiosis risk (Cumming, 1994) and the risk of feed-borne pathogen invasion (Engberg *et al.,* 2002). Particle size distribution of the feed has been reported to affect the growth of various other sections of the digestive tract in birds maintained on mash diets. Nir *et al.* (1994) concluded that the small intestine showed signs of hypertrophy and lower pH readings were recorded when birds were maintained on mash diets with a fine particle size distribution. Likewise, a comparable pattern was recorded in birds maintained on whole-wheat diets (Gabriel *et al.,* 2003). The effect of lower duodenal weights linked with diets consisting of coarse feed particles is uncertain.

Table 2.3 Influence of particle size and feed form on gross morphology of the digestive tract of broilers maintained on wheat-based pelleted and mash diets (Amerah *et al.*, 2018)

	Mash		**Pellets**	
Item	Medium	Coarse	Medium	Coarse
Relative empty weight (g/kg of BW)				
Crop	3.1 ± 0.29	2.9 ± 0.15	3.2 ± 0.28	3.0 ± 0.13
Proventriculus	5.8 ± 0.31	5.1 ± 0.21	5.2 ± 0.46	5.5 ± 0.65
Gizzard	22.0 ± 0.74	20.1 ± 0.71	10.7 ± 0.66	11.3 ± 0.72

2.4.3. Digesta particle size distribution

Very few studies have been conducted on the effect of the particle size distribution on the particle size distribution in the digesta of poultry. Hetland *et al.* (2004) concluded that the gizzard can grind almost all natural ingredients of feed to a consistent fine texture irrespective of the original particle size of the feed (Hetland *et al.,* 2002).

In contradiction, Lentle (2005) speculated that a higher percentage of coarser particles in the feed resulted in bigger quantities of coarser particles to transfer through the gizzard into the rest of the GIT, but that these coarser particles improved the digestion efficiency due to improved permeability of the digesta to digestive enzymes. This assumption was founded on the fact that the digesta contained a combination of coarse and fine particles, with the fine particles inhabiting the small areas between the coarser particles, which caused a decrease in the mean void space radius accessible for permeation (Wise, 1952). Consequently, a higher percentage of coarser particles might lead to a local rise in permeation of digestive fluids through sites where there is a higher percentage of coarser particles (Lentle, 2005; Lentle *et al*., 2006).

More recently (Amerah *et al.,* 2007c) concluded that gizzard weights were higher in birds maintained on mash diets and that the duodenal digesta contained a higher percentage of coarser particles (1000-2000 μm) vs birds maintained on pelleted diets. Even though the gizzard size increased in response to a higher proportion of coarse particles in the diet, its particle size reduction (grinding) was inconsistent. The improved starch digestibility related to improved gizzard development of the birds maintained on diets comprising complete cereals (Hetland *et al.,* 2002) can be a result of changes in the permeability from a hidden rise in the percentage of coarse particles.

2.4.4. Passage rate

It is said a slower passage rate can inhibit feed intake, but a faster passage rate limits the time presented for digestion and absorption (Svihus *et al.,* 2002). The digesta passage rate is typically measured with insoluble coloured markers for instance, chromic or ferric oxide. However, it must be noted that results can be perplexed by the retention of specific particle sizes in certain sections of the GIT, by cohesion to other particles or through dissolution (Svihus *et al.,* 2002). Also, in some species, soluble nutrients flow through the gut at a higher rate than most particles (Lentle, 2005). Solid phase markers show in the excreta 1.6 to 2.6 hr after consumption (Denbow, 2000). Numerous factors like the broiler strain, physiological age, amount of non-starch polysaccharides in the diet, the water insoluble non-starch polysaccharides inclusion level, fat levels in the diet and environmental temperature can influence the passage rate of solid phase markers (Denbow, 2000). Normally, bigger particles tend to remain in the digestive tract for longer periods than finer particles (Denbow, 2000), increasing the mean residence time. Therefore, the amount of coarser particles in the gizzard may be twice as much as in the ingested feed (Hetland *et al.,* 2004) perhaps due to selective retention of coarser particles (Hetland *et al.,* 2004). Yet, the total retention time will not be longer for birds maintained on whole grains (Svihus *et al*., 2002; Svihus *et al.,* 2004). Hetland *et al.* (2005) hypothesized that there is quick dissipation of the starch granule and protein of whole grains in the gizzard where there is a low pH, quickly reducing the particle size of the whole grains without affecting the passage rate.

2.4.5. Nutrient utilisation

Even though a smaller particle size distribution can boost nutrient digestion by providing a bigger surface area to the digestive enzymes, studies which compares particle size distribution to nutrient digestibility are restricted and very unclear, specifically for grains. Kilburn & Edwards (2001) concluded that finer maize particles improved the true metabolisable energy values in diets fed as mash, but the contradictory effect was detected when the feed was in the pelleted form. Peron *et al.* (2005) showed that wheat with a fine particle size had better starch digestibility and apparent metabolisable energy (AME) vs wheat with a bigger particle size distribution. In contrast, maize with a bigger particle size distribution may improve the efficiency of nitrogen and lysine retention in broilers on mash diets (Parsons *et al.,* 2006).

Amerah *et al.* (2007b) concluded that wheat in diets that was coarsely ground had a better AME value, however, in maize-based diets this was not seen. In contradiction, Svihus *et al.* (2004) found that the AME was not influenced by the particle size of wheat. There is negative correlation between the hardness of wheat and the starch digestibility in pelleted diets (Carre *et al.,* 2002). This harder wheat resulted in coarser particles after milling, subsequently reducing the surface area and availability to digestive enzymes (Carre *et al.,* 2002). In contrast, Uddin *et al.* (1996) concluded that the endosperm hardness of wheat had no effect on the AME in pelleted diets. Inconsistency in the correlation between grain hardness and AME or starch digestibility in wheat-based mash diets has been reported by other studies (Rogel *et al.,* 1987).

The findings for dicotyledonous seeds are more consistent. Legumes that were coarsely ground revealed lower energy utilisation and nutrient digestibility coefficients. The effects of particle size on starch digestibility in grain legumes have been reviewed and are available (Carre, 2004). Generally, peas that were finely ground had better total tract digestibility of starch and protein when the birds were maintained on mash diets (Carre, 2000). Likewise, finely ground peas also had better apparent ileal protein digestibility (Crevieu *et al.,* 1997). The same positive effects were seen for fine sweet lupine seeds (Crevieu *et al.,* 1997). These results may be due to the nutrients being more accessible in the finely ground legume seeds. In contrast, finely ground faba beans didn't result in better total tract digestibility of protein (Lacassagne *et al.,* 1991).

The inconsistent conclusions on the influence of feed particle size on nutrient digestibility may be due to differences in where the measurement is taken (ileal digesta compared to excreta). The inconsistent and altering effects of microflora in the caeca on protein digestibility was accepted more recently and it is now commonly accepted that ileal digesta should be analysed instead of excreta when measuring nutrient digestibility in poultry (Ravindran & Bryden, 1999).

Coarse grinding may hold some advantages over fine grinding when analysing mineral availability. Maize with a bigger particle size distribution had a significantly better calcium, total phosphorus and phytate phosphorus utilisation in broilers (Kilburn & Edwards, 2001). Bone ash and plasma phosphorus levels of broilers receiving coarsely ground soybean meal were significantly better (GMD, 1239 µm) compared with finely ground (GMD, 891 µm) soybean meal (Kilburn & Edwards, 2004). Comparable conclusions were drawn with maize-soya based diets by Carlos & Edwards (1997). It was speculated that bigger particles resulted in longer retention time permitting more time for mineral digestion and absorption. These advantages were decreased when feed was presented pelleted or crumbled form (Kilburn & Edwards, 2004), apparently due to the dissociation of the bigger particles throughout the pelleting process.

Very limited literature is available on the effect of different particle size distributions on the precaecal crude protein (CP) and amino acid digestibility in broilers. Siegert *et al.* (2017) concluded that the particle size of the feed constituents affects the precaecal CP and AA digestibility and that this effect may differ for different feed constituents. The precaecal CP and AA digestibility and thus broiler performance was significantly better for soybean meal and was numerically, yet insignificantly, reduced in maize by decreasing the screen size of the hammer mill from 3 to 2 mm (Siegert *et al.*, 2018). The numerical value of the difference in precaecal AA digestibility when comparing the particle sizes was 0.055% in soybean meal (lowest value being 0.035% for arginine to the highest value of 0.083% for proline) (Siegert *et al.*, 2017). This value was comparable in maize, typically being around 0.045% (lowest value being 0.020% for methionine and the highest value being 0.082% for serine) (Siegert *et al.*, 2017). The standard errors in the digestibility estimates of maize were significantly bigger than for soybean meal. This resulted in the absence of significant differences between the different particle size distributions of maize. Table 2.4 shows the performance parameters of this study and Table 2.5 shows the precaecal CP an AA digestibility.

Table 2.4 The effect of growth performance, intestine length and digesta quantity in broilers (Siegert *et al.*, 2018)

Diet	1	2	3	4	5	6	7	8	9	
Test ingredient	Maize					Soybean meal				
Grid size		2 mm	2 mm	3 mm	3 mm	2 mm	2 mm	3 mm	3 mm	
Inclusion level	0	250	500	250	500	150	300	150	300	Pooled SEM
Body weight day 21 (g)	821^{e}	880^{d}	967^{bc}	889^{d}	935^{cd}	982^{ab}	1002^{a}	944^{c}	984^{ab}	12.2
Feed intake (g/Bird)	80^{d}	85^{c}	94^{a}	89^{bc}	91^{ab}	92^{ab}	93^{ab}	90^{b}	95^{a}	1.30
Intestine length [1] (cm)	59^{b}	61^{ab}	64^{a}	63^{a}	63^{a}	64^{a}	65^{a}	63^{a}	65^{a}	0.90
Digesta [2] (g/bird)	1.1^{d}	1.5^{bc}	1.9^{a}	1.6^{abc}	1.7^{ab}	1.3^{c}	1.7^{ab}	1.3^{c}	1.7^{ab}	0.07

$^{a-e}$Means within a row without a common superscript differ significantly ($p<0.05$)

[1] Segment of the small intestine between Meckel's diverticilum to the region 2 cm anterior to the ileocaeca-colonic junction

[2] Freeze-dried material obtained from the terminal two-third region of the small intestine between Meckel's diverticulum and the region 2 cm anterior to the ileocaeca-colonic junction.

SEM: Standard error of the mean

Table 2.5 Precaecal protein and amino acid digestibility (%) of maize and soybean meal of different particle sizes when fed to broilers (Siegert *et al.*, 2018)

Diet	1	2	3	4	5	6	7	8	9	
Test ingredient	Maize					Soybean meal				
Grid size		2 mm	2 mm	3 mm	3 mm	2 mm	2 mm	3 mm	3 mm	
Inclusion level	0	250	500	250	500	150	300	150	300	Pooled SEM
Crude Protein	0.88^{a}	0.86^{ab}	0.84^{d}	0.86^{abc}	0.85^{bcd}	0.86^{abc}	0.82^{e}	0.85^{cd}	0.80^{f}	0.006
Alanine	0.82^{a}	0.82^{a}	0.79^{c}	0.82a	0.81^{ab}	0.82^{a}	0.77^{cd}	0.80^{abc}	0.74^{d}	0.010
Arginine	0.92^{a}	0.91^{a}	0.90^{b}	0.91^{ab}	0.91^{ab}	0.91^{ab}	0.88^{c}	0.90^{b}	0.86^{d}	0.004
Asparagine	0.77^{ab}	0.77^{ab}	0.73^{cd}	0.76^{b}	0.76^{bc}	0.80^{a}	0.75^{bcd}	0.77^{ab}	0.72^{d}	0.010
Cysteine	0.75^{a}	0.74^{ab}	0.72^{bc}	0.74^{ab}	0.73^{ab}	0.73^{abc}	0.65^{d}	0.70^{c}	0.61^{d}	0.009
Glutamine	0.94^{a}	0.93^{b}	0.90^{d}	0.93^{b}	0.91^{cd}	0.92^{bc}	0.88^{e}	0.91^{cd}	0.87^{f}	0.004
Glycine	0.79^{ab}	0.78^{ab}	0.75^{d}	0.77^{abc}	0.76^{bcd}	0.80^{a}	0.75^{cd}	0.77^{abc}	0.71^{e}	0.008
Isoleucine	0.90^{a}	0.89^{ab}	0.86^{d}	0.89^{abc}	0.88^{bcd}	0.89^{abc}	0.84^{e}	0.86^{cd}	0.82^{e}	0.006
Leucine	0.89^{a}	0.88^{ab}	0.85^{cd}	0.88^{ab}	0.87^{bc}	0.88^{ab}	0.83^{de}	0.86^{bc}	0.80^{e}	0.007
Lysine	0.92^{a}	0.91^{ab}	0.89^{cd}	0.91^{ab}	0.90^{bc}	0.90^{bc}	0.87^{d}	0.89^{cd}	0.85^{e}	0.005
Methionine	0.95^{a}	0.94^{ab}	0.93^{cd}	0.94^{abc}	0.94^{bcd}	0.94^{abcd}	0.91^{ef}	0.92^{de}	0.90^{f}	0.005
Phenylalanine	0.89^{a}	0.88^{ab}	0.85^{cd}	0.88^{ab}	0.87^{bc}	0.88^{ab}	0.83^{de}	0.87^{bc}	0.81^{e}	0.007
Proline	0.91^{a}	0.89^{bc}	0.87^{de}	0.89^{bc}	0.88^{cd}	0.91^{ab}	0.85^{e}	0.89^{bc}	0.82^{f}	0.006
Serine	0.83^{a}	0.83^{a}	0.78^{c}	0.82^{ab}	0.82^{ab}	0.84^{a}	0.79^{bc}	0.81^{ab}	0.76^{c}	0.009
Threonine	0.86^{a}	0.85^{ab}	0.82^{c}	0.85^{ab}	0.84^{ab}	0.85^{ab}	0.80^{c}	0.83^{bc}	0.77^{d}	0.007
Valine	0.91^{a}	0.89^{ab}	0.87^{d}	0.89^{abc}	0.88^{cd}	0.89^{bcd}	0.84^{e}	0.87^{d}	0.81^{f}	0.006

$^{a\text{-}f}$Means in a row with different superscripts differ significantly ($p < 0.05$)

SEM: Standard error of the mean

2.4.6. Microbiome

Overall gut health and consistency of the intestinal tract environment is a composite occurrence, depending on a fragile balance linking the dietary constituents, the commensal microbiome and the tract mucosa (Montagne *et al*., 2003). The GIT consists of a condensed and diverse microbial community. Resistance against in-feed antibiotics gave rise to increased attention towards supporting the balance between advantageous bacteria, like *Lactobacillus, Enterococcus, Bifidobacterium* spp., and possible pathogenic bacteria, such as *Clostridium perfringens, Escherichia coli* and *Salmonella* spp. (Matin *et al.,* 2012) in the GIT. Diet constituents are key in controlling the structure, balance and metabolic function of microbiota throughout the GIT (Sonnenburg & Bäckhed, 2016).

Numerous studies have shown that particle size distribution of the diet affects the gut microflora. Birds that are maintained on a diet consisting of a finer particle size distribution will have a faster passage rate into the duodenum which results in the digesta being exposed to the enzymes and low pH in the gizzard for a much shorter time. (Hill, 1971). Therefore, a relatively bigger proportion of undigested particles are found in the upper small intestine, which can cause greater pathogenic bacterial populations, like *Clostridium perfringens* and *E. coli* (Cumming, 1994). Providing birds with a diet containing coarsely ground grains promotes the grinding activity in the gizzard which prolongs the digesta retention time in the fore-gut and the crop (Svihus, 2011). In that way, stimulating the process of fermentation, owing to the occurrence of bacteria of the *Lactobacilli*, *Streptococci* and *Coliform* spp. inside the crop (Fuller, 2001). This may improve the spreading of these beneficial bacteria within the intestine and hindgut of poultry.

Various studies concluded that coarsely ground grain in the diet of poultry resulted in higher levels of beneficial bacteria like *Lactobacillus* and *Bifidobacteria* spp. in the caeca and reduction in the amount of pathogenic bacteria like *Clostridium*, *Campylobacter, Escherichia* and *Salmonella* spp. (Engberg *et al*., 2002; Jacobs *et al.,* 2010). High levels of beneficial bacteria can improve the native and acquired immune responses in poultry (Calixto *et al*., 2004). Thus, the inclusion of coarse particles in poultry diets may result in improved intestinal health, which can be a valuable tool during the movement away from antibiotic use in the feed.

2.5. Conclusion

This review focused on the studies done concerning the optimum particle size of various feed constituents for efficient poultry production. The present feed manufacturing practice of using highly processed, pelleted diets, seemingly disguises the effects of particle size on overall performance. Existing data show that the particle size distribution of grains is more important in mash based diets than in pelleted or crumbled diets. Even though it is commonly thought that finer grinding will provide a bigger, and coarse grinding will provide a smaller surface area available for enzymatic digestion, it is suggested that more uniform coarser particles enhances the overall production performance of broilers. This contradictory effect can be due to a better developed gizzard when birds are fed a diet consisting of coarser particles. A completely developed gizzard is linked with improved grinding ability, consequently improving gut motility and nutrient digestion, but also resulting in smaller particles flowing into the small intestine and improving the availability of the digesta to the digestive enzymes. It is important to keep GMD and GSD in mind when studying particle size as they are vital to maintain good bird performance. There is a lot of information available on maize particle size but hardly any information on the optimum particle size of the other feed ingredients, such as soybean meal.

The effect of the particle size distribution of feed ingredients on pellet quality are not well understood. Additional methodical research regarding the balance between feed particle size distribution, broiler production performance, gut health and pellet quality is required if production efficiency is to be optimised with respect to the energy expenditure of grinding.

Chapter 3

Materials and Methods

3.1. Ethical approval

This trial was approved by the Faculty of Natural and Agricultural Sciences, University of Pretoria with reference number NAS028/2019.

3.2. Experimental design

Two broiler houses, each consisting of 64 pens, were used in the trial. A complete randomised block design was utilised. Each house was divided into five blocks, and therefore the trial had ten blocks in total.

There were 12 treatments in the trial, each replicated 10 times. Each replicate appeared once in each of the 10 blocks and comprised of a pen containing 60 broilers. Treatments were different combinations of maize and soybean meal particle sizes as shown in Table 3.1 and discussed in section 3.4.

3.3. Housing and management

The trial was run at the test facilities at Daybreak Farms, Sundra, South Africa. Broilers were housed in a standard open-sided broiler house, fitted with tunnel ventilation. This house was divided into two separately controlled sides. Seven thousand two hundred (7200) vaccinated day-old Ross 308 chicks were purchased from the Daybreak Merinovlakte hatchery. On arrival at the trial house, a total of 60 chicks were randomly selected and allocated to one of 120 pens (60 pens per side). Each pen had an area of 3 m^2, however, the two tube feeders used occupied an area of 0.407 m^2, resulting in a total usable area for the chicks of 2.593 m^2. Thus, the chicks were placed at a stocking density of 23 birds per m^2. The temperature profile that was followed from 2 days pre-placement to Day 35 is shown in appendix A; the lighting profile is shown in appendix B and the vaccination program is shown in appendix C. The bedding consisted of pine shavings, approximately 10 cm deep. Each pen contained two tube feeders and seven nipple drinkers.

The birds were monitored daily by the principal investigator and trial farm staff. There were farm personnel on the premises at all times throughout the trial to monitor the birds' comfort regarding heat, ventilation, feed and water supply, as well as general health. Temperature and humidity loggers were installed in both sides at the beginning of the trial to ensure maximum comfort was maintained for the birds throughout the trial. The birds had *ad libitum* access to feed and water at all times.

3.4. Dietary treatments

The birds were fed on a three-phase feeding scheme which included a crumbled Starter, pelleted Grower and pelleted Finisher which was fed for 17, 10, and 8 days respectively. Starter was weighed back and discarded on Day 17, Grower was weighed back on Day 27, and Finisher was weighed back on Day 35. The birds were fed according to the number of days on feed and feed was weighed out, recorded and then discarded for each treatment. The feeders were kept full at all times to avoid risk of restricted feed intake. Table 3.1 indicates the different treatments.

Table 3.1 Treatments with different combinations of maize and soybean meal particle sizes

Treatment	Maize [#] (% coarse)	Soybean meal (screen size)[$]	Number of replicates / treatment
T1	15% coarse	unmilled	10
T2	15% coarse	6 mm	10
T3	15% coarse	4 mm	10
T4	15% coarse	2 mm	10
T5	30% coarse	4 mm	10
T6	30% coarse	6 mm	10
T7	40% coarse	6 mm	10
T8	50% coarse	6 mm	10
T9	0% coarse (100% fine)	unmilled	10
T10	50% coarse	2 mm	10
T11	0% coarse (100% fine)	2 mm	10
T12	50% coarse	unmilled	10

[#] Coarse maize was defined as maize where at least 60% of the particles were larger than 1.4 mm in diameter

[$] The size given in mm refers to the size of the screen on the roller mill on which the soybeans were milled

The fine maize in these treatments was defined as maize where at least 85% of the particles were smaller than 1.4 mm in diameter, thus no more than 15% of the particles were larger than 1.4 mm. The percentage of maize given in Table 3.1 per treatment refers to a percentage of the maize fraction of the diet and not as a percentage of the total diet.

For the soybean meal, the size given in mm refers to the size of the screen on the roller mill on which the soybeans were milled. The exact particle fractions of each of the sizes is set out in Table 3.5. Three soybean samples were randomly taken with a probe for each of the screen sizes and then the average percentage was calculated for each fraction. The 2 mm screen soybean meal can be defined as soybean meal where 72.3% of the particles were smaller than 1.18 mm, whereas the 4 mm screen soybean meal can be defined as soybean meal where 63.9% of the particles were smaller than 1.18 mm. The 6 mm screen soybean meal can be defined as soybean meal where 58.7% of the particles were smaller than 1.18 mm and lastly the unmilled soybean meal can be defined as soybean meal where only 44.1% of the particles were smaller than 1.18 mm.

Unmilled soybean meal refers to soybean meal as received by AFGRI Animal Feeds Isando mill (Gauteng) from the suppliers (mainly Nedan, Limpopo). This unmilled soybean meal contains various particles of bigger than 6 mm in diameter. The treatments which contain unmilled soybean meal were included to see whether it is actually necessary to further mill the soybean meal, or can it just be used as received. Not having to further mill the soybean meal will bring forth a massive cost saving during the production process. This unmilled soybean meal where tested in combination with 15% coarse maize, 50% coarse maize and 100% fine maize. The combination with 15% coarse maize (treatment 1) was included because 15% coarse maize was used in AFGRI's commercial broiler rations at the time of the trial. The 50% combination (treatment 12) was included to test whether the coarse material will cause better gizzard and proventriculus development and thus better performance. Lastly, the combination with 100% fine maize (treatment 9) was included to determine whether the coarse soybean meal particles would sufficiently stimulate gizzard and proventriculus development.

The 6 mm soybean meal screen size was combined with different inclusion levels of coarse maize in order to test the effect of increasing inclusion levels of coarse maize in the diet on overall broiler performance, gizzard and proventriculus development and pellet durability. This refers to treatment 2 (15% coarse maize), treatment 6 (30% coarse maize), treatment 7 (40% coarse maize) and treatment 8 (50% coarse maize).

Treatments 3 and 5 both contained soybean meal milled through a 4 mm screen with different combinations of coarse maize inclusion levels. Treatment 3 included 15% coarse maize and treatment 5 included 30% coarse maize to once again compare different inclusion levels of coarse maize on overall broiler performance, gizzard and proventriculus development and pellet durability.

Treatment 4, 10 and 11 contained soybean meal milled on a 2 mm screen. Treatment 11 contained no coarse maize (100% fine maize) and soybean meal from a 2 mm screen in order to test whether or not coarse particles are actually essential in a pelleted diet. Treatment 4 contained 15% coarse maize and treatment 10 contained 50% coarse maize to again test how much coarse maize is needed in a pelleted diet when the soybean meal is milled in a 2 mm screen.

Treatment 2, 3 and 4 all contained 15% coarse maize with soybean meal of different screen sizes in order to test the effect of soybean meal particle size on overall broiler performance, gizzard and proventriculus development and pellet durability. Treatment 2 contained soybean meal milled on a 6 mm screen, treatment 3 contained soybean meal milled on a 4 mm screen and treatment 4 contained soybean meal milled on a 2 mm screen.

All the treatments (treatment 1-12) were be compared with each other in order to identify the optimum maize and soybean meal particle size for optimal broiler performance, gizzard and proventriculus development and pellet durability.

All the different coarsenessess of soybean meal and maize were analysed through near-infrared reflectance (NIR) spectroscopy to determine the protein, moisture and fat content. These values were then used during formulation to ensure that all the treatments had the same nutrient and moisture levels. This ensured that the nutrient and especially the protein levels remained constant throughout all the different treatments and that the only difference between the treatments were the particle sizes of maize and soybean meal.

Diet constituents, inclusion levels and calculated specifications for each phase feeds are shown in Tables 3.2, 3.3 and 3.4.

Table 3.2 Raw material inclusion (%) and calculated nutrient composition for starter trial feed

Raw material	**Inclusion %**
Yellow Maize	58.89
Soya Oilcake Meal 46	27.40
Sunflower Oilcake CF 20-24 CP ≥ 38	3.000
Carcass Meal	4.433
Synthetic Valine	0.012
Synthetic Lysine	0.185
Synthetic Methionine	0.220
Synthetic Tryptophan	.
Synthetic Threonine	0.050
Soya Oil (Mixer)	2.167
Soya Oil (Coater)'	.
Limestone (Savanna)'	1.177
Salt fine	0.433
FORMI® (ADDCON 40% formic acid product)	.
Olaquindox (10%)	0.040
Choline Chloride Liquid LM (75%)	0.067
Cycostat® (Zoetis robenidine hydrochloride product)	0.050
Mycofix® Select (BIOMIN mycotoxin binder)	0.100
Hemicell® HT (Elanco mannanase product)	0.033
Monocalcium Phosphate	1.093
CreAMINO® (Philagro guanidinoacetate product)	0.060
Digestarom® (BIOMIN phytogenic supplement)	0.037
Lysine sulphate 70% (55% true lysine)	0.240
Axtra® XAP/Axtra® Phy2000FTU (Chemuniqué xylanase, amylase and protease/phytase blend)	0.050
Natuphos® 1000 FTU broiler (BASF phytase product)	0.010
Calculated Nutrient Composition (%)	
Dry Matter	88.03
AME (MJ.kg^{-1})[1]	12.75
Moisture	11.69
Crude Protein	22.34
Crude Fat	4.650
Crude Fibre	3.610
Ash	5.250
Calcium	0.860
Phosphorus	0.620
Available Phosphorus	0.330
Lysine (Total)	1.280
Methionine (Total)	0.540

[1] AME (apparent metabolisable energy) for broiler chicks (CVB, 2018)

Table 3.3 Raw material inclusion (%) and calculated nutrient composition for grower trial feed

Raw material	**Inclusion %**
Yellow Maize	59.54
Soya Oilcake Meal 46	20.50
Sunflower Oilcake CF 20-24 CP ≥ 38	4.500
Carcass meal	8.900
Synthetic Valine	0.015
Synthetic Lysine	0.285
Synthetic Methionine	0.205
Synthetic Tryptophan	0.015
Synthetic Threonine	0.044
Soya Oil (Mixer)	1.300
Soya Oil (Coater)'	1.500
Limestone (Savanna)'	0.990
Salt fine	0.350
FORMI® (ADDCON 40% formic acid product)	.
Olaquindox (10%)	0.040
Choline Chloride Liquid LM (75%)	0.067
Cycostat® (Zoetis robenidine hydrochloride product)	0.050
Mycofix® Select (BIOMIN mycotoxin binder)	0.100
Hemicell® HT (Elanco mannanase product)	0.033
Monocalcium Phosphate	0.921
CreAMINO® (Philagro guanidinoacetate product)	0.060
Digestarom® (BIOMIN phytogenic supplement)	0.037
Lysine sulphate 70% (55% true lysine)	0.240
Axtra® XAP/Axtra® Phy2000FTU (Chemuniqué xylanase, amylase and protease/phytase blend)	0.050
Natuphos® 1000 FTU broiler (BASF phytase product)	0.010
Calculated Nutrient Composition (%)	
Dry Matter	88.18
AME (MJ.kg^{-1})[1]	13.17
Moisture	11.55
Crude Protein	21.47
Crude Fat	5.560
Crude Fibre	3.750
Ash	4.630
Calcium	0.770
Phosphorus	0.570
Available Phosphorus	0.290
Lysine (Total)	1.220
Methionine (Total)	0.510

[1] AME (apparent metabolisable energy) for broiler chicks (CVB, 2018)

Table 3.4 Raw material inclusion (%) and calculated nutrient composition for finisher trial feed

Raw material	**Inclusion %**
Yellow Maize	59.49
Soya Oilcake Meal 46	21.57
Sunflower Oilcake CF 20-24 CP ≥ 38	5.000
Carcass meal	6.667
Synthetic Valine	0.010
Synthetic Lysine	0.237
Synthetic Methionine	0.194
Synthetic Tryptophan	.
Synthetic Threonine	0.037
Soya Oil (Mixer)	1.900
Soya Oil (Coater)'	2.167
Limestone (Savanna)'	0.900
Salt fine	0.327
FORMI® (ADDCON 40% formic acid product)	.
Olaquindox (10%)	.
Choline Chloride Liquid LM (75%)	0.067
Cycostat® (Zoetis robenidine hydrochloride product)	.
Mycofix® Select (BIOMIN mycotoxin binder)	0.100
Hemicell® HT (Elanco mannanase product)	0.033
Monocalcium Phosphate	0.704
CreAMINO® (Philagro guanidinoacetate product)	0.060
Digestarom® (BIOMIN phytogenic supplement)	0.037
Lysine sulphate 70% (55% true lysine)	0.240
Axtra® XAP/Axtra® Phy2000FTU (Chemuniqué xylanase, amylase and protease/phytase blend)	0.050
Natuphos® 1000 FTU broiler (BASF phytase product)	0.010
Calculated Nutrient Composition (%)	
Dry Matter	88.30
AME (MJ.kg^{-1})[1]	13.50
Moisture	11.48
Crude Protein	21.29
Crude Fat	6.630
Crude Fibre	3.870
Ash	4.350
Calcium	0.700
Phosphorus	0.520
Available Phosphorus	0.240
Lysine (Total)	1.200
Methionine (Total)	0.500

[1] AME (apparent metabolisable energy) for broiler chicks (CVB, 2018)

Table 3.5 The percentage per sieve compartment of the soybean meal samples milled on different screen sizes

	Sieve screen sizes								
Roller mill screen size	**>3.55 mm**	**2.50 - 3.55 mm**	**2.36 - 2.49 mm**	**2.0 - 2.35 mm**	**1.4 - 1.99 mm**	**1.18 - 1.39 mm**	**1.0 - 1.17 mm**	**<1.0 mm**	**Total <1.18 mm**
Average 2 mm	0	0	0	0.1	15	12.6	11.2	61.1	72.3
Average 4 mm	0	0.4	0.2	3.5	17.5	14.5	10.5	53.4	63.9
Average 6 mm	0.1	1.6	0.5	4.2	19.7	15.2	7.9	50.8	58.7
Average Unmilled	0.7	3.6	0.7	6.6	25.1	19.2	4.1	40	44.1

Analyses of feed samples for each phase were conducted using near infrared spectroscopy (NIRS; Quality Control Laboratory, AFGRI Animal Feeds, Isando) before commencement of the trial to ensure that the feeds within each phase contained the same nutrient composition. Table 3.6 – 3.8 show the nutrient content of the three phase feeds for each of the treatments.

Table 3.6 Near-infrared reflectance spectroscopy results (%) obtained for the starter phase of each treatment

Treatment	Crude Protein	Moisture	Fat
T1	20.5	10.8	4.71
T2	20.7	10.7	4.78
T3	20.4	10.8	4.85
T4	20.6	10.6	4.68
T5	20.4	10.9	4.88
T6	20.9	10.8	4.77
T7	20.5	10.7	4.91
T8	20.7	10.8	4.86
T9	20.2	10.6	4.79
T10	20.4	10.9	4.81
T11	20.6	10.7	4.72
T12	20.3	10.7	4.90

Table 3.7 Near-infrared reflectance spectroscopy results (%) obtained for the grower phase of each treatment

Treatment	Crude Protein	Moisture	Fat
T1	20.8	10.3	4.55
T2	20.1	10.2	4.58
T3	20.3	10.4	4.55
T4	20.7	10.3	4.57
T5	20.6	10.3	4.56
T6	21.0	10.5	4.52
T7	19.9	10.2	4.52
T8	19.8	10.1	4.53
T9	20.8	10.1	4.55
T10	20.4	10.4	4.59
T11	20.6	10.3	4.62
T12	20.5	10.6	4.52

Table 3.8 Near-infrared reflectance spectroscopy results (%) obtained for the finisher phase of each treatment

Treatment	Crude Protein	Moisture	Fat
T1	20.1	10.9	6.53
T2	20.3	10.7	6.58
T3	20.6	11.2	6.55
T4	19.9	11.1	6.57
T5	20.8	11.1	6.56
T6	20.6	11.0	6.58
T7	20.4	11.1	6.52
T8	20.7	10.9	6.51
T9	20.4	10.8	6.55
T10	19.9	11.1	6.57
T11	20.4	11.2	6.49
T12	20.5	11.1	6.48

3.5. Performance measurements

3.5.1 Bodyweight (BW)

Broilers were weighed weekly to obtain average bodyweight (BW) for each individual pen. All the birds in a pen were weighed collectively in a crate, which was tared before every weighing, and the average BW was then calculated by dividing the recorded value by the number of birds in the pen. The day-old chicks were weighed at placement and then again at 7, 14, 21, 28 and 35 days-of-age.

3.5.2 Feed intake (FI)

Weekly feed intake (FI) was measured by weighing out a specific amount at the beginning of the phase and weighing the feed that was left over at the end of the week. The weekly weighing of FI occurred at the same time as the weighing of the birds. Cumulative feed intake was calculated by the summation of the weekly feed intakes.

3.5.3 Feed conversion ratio (FCR)

The cumulative feed conversion ratio (FCR) was calculated by dividing the cumulative FI of the pen by the total BW gained per pen over the experimental period and was corrected for mortality by adding the bodyweight gain of the mortalities during the week to the bodyweight gain of the pen during the week.

3.5.4 European performance efficiency factor (PEF)

The European performance efficiency factor (PEF) value is a calculated value incorporating all of the performance factors and is regarded as a good measure of overall performance, for commercial purposes. The following equation was used:

PEF = (Liveability % x Mass (kg)/ Age in Days x FCR) x10.

3.5.5 Mortalities

The trial house was inspected twice daily; any mortalities were removed, weighed and recorded.

3.6. Gizzard and proventriculus measurements

One bird per pen was sacrificed and slaughtered on day 7, 14 and 35, respectively. These birds were euthanised humanely via cervical dislocation and then the gizzard and proventriculus were surgically removed, washed and weighed with the kaolin layer intact. The gizzard and proventriculus, as well as bird weight, were recorded in order to calculate the gizzard and proventriculus weight as a % of bodyweight.

3.7. Pellet hardness measurement

Three samples were randomly taken from three different 40 kg bags of feed of each treatment, respectively. Fifty pellets from each sample bag (150 pellets per treatment) of the grower and finisher phase were tested for hardness using the Kahl pellet hardness tester. A single pellet was placed in the abovementioned device and the force on the pellet was gradually increased until the pellet breaks. These hardness measurements were then recorded.

3.8. Statistical analysis

The trial was established in two broiler houses as a randomised complete block design to test for differences between twelve treatment effects with five blocks per house. Randomised complete block design analysis of variance (ANOVA) on the growth and slaughter data was used to test for differences between treatment effects. After analysis, the standardised residuals were found to be acceptably normal distributed (Shapiro & Wilk, 1965) and treatment variances were homogeneous.

The average pellet hardness data (from three samples analysed) was analysed with complete randomised design ANOVA to test for treatment effects, but at the 1% level as the treatments variances were not homogeneous.

Treatment means of the significant effects were compared using Fisher's protected least significant difference (LSD) test at the 5% ($p < 0.05$) or 1% ($p < 0.01$) level of significance. Means followed by different upper-case letters were significantly different at the 5% or 1% level (Freund, Mohr & Wilson, 2010). All data analyses were performed using the GenStat® statistical software (VSN International, 2017).

Chapter 4

Results and discussion

4.1. The effect of maize and soybean particle size on broiler bodyweight

It is common belief that the major effect of the particle size of feed will be eliminated if the feed is offered in a crumble or pelleted form. Nir *et al.* (1995) suggested that the pellet will eventually dissolve in the crop after consumption, thus maintaining the effect of particle size even when the feed is given in the pelleted form. Various studies in the past found that particle size had no effect on performance of broilers that were given a pelleted diet. No significant effect was found on broiler performance when maize of varying particles sizes was used to formulate a starter feed for broilers (Reece *et al.*, 1986). Svihus *et al.* (2004a) also concluded that the pelleting of the diet eliminated the effect of particle size distribution in the feed. Even though the differences in particle size was retained after pelleting, Peron *et al.* (2005) found that pelleted broiler diets made of wheat of two differing particle sizes had no effect on overall broiler performance.

Lentle *et al.* (2006) concluded that diets containing a higher percentage of coarse maize particles resulted in improved feed efficiencies. In contrast to this, Lott *et al.* (1992) found that broilers fed pelleted diets containing a higher percentage of coarse maize gave rise to reduced weight gain and feed efficiency when compared to pelleted diets containing more finely ground maize. This was confirmed by Kilburn & Edwards (2001) when they concluded that broiler performance was improved when the broilers were fed pelleted diets containing finely ground maize instead of only coarse maize. This shows the lack of consistency in the literature available on the topic of particle size in pelleted diets.

Table 4.1 shows the bodyweight of the broilers from day 0 to 35. In contrast to the findings of Reece *et al.* (1986) and Svihus *et al*. (2004a), this study found that there was a definite and significant effect of particle size on broiler performance in both crumbled and pelleted diets. From day 7 to 35 there was a significant difference in the bodyweight (BW) of broilers fed on treatment 1 (15% coarse maize; unmilled soybean meal), treatment 12 (50% coarse maize; unmilled soybean meal) and treatment 9 (100% fine maize; unmilled soybean meal). With unmilled soybean meal in the diet, the highest BW from day 7 to 35, was obtained when the maize fraction of the diet contained 15% coarse particles.

Siegert *et al.* (2018) found that the precaecal CP and AA digestibility and thus broiler performance was significantly increased in soybean meal by reduction of the grid size of the hammer mill from 3 to 2 mm. The results obtained from this study shows that there was a significant difference in the BW from day 7 to 35 when comparing treatment 2 (15% coarse maize; 6 mm soybean meal), treatment 3 (15% coarse maize; 4 mm soybean meal) and treatment 4 (15% coarse maize; 2 mm soybean meal). From day 7 to 14 it was found that 6mm soybean meal resulted in the highest BW. But in contrast to this, from day 21 to 35, as the particle size of the soybean meal was reduced, the BW of the broilers increased significantly which agrees with the conclusions of Siegert *et al.* (2018).

Nir *et al.* (1994) found that from day 0 to 7 particle size had no significant effect on performance of broilers, but broilers fed diets containing more coarse maize did perform better from day 7 to 21. This is due to young birds not having well developed gizzards before 7 days-of-age. This study found that from day 7 to 35 there were significant differences in the BW of broilers fed on treatment 2 (15% coarse maize; 6 mm soybean meal), treatment 6 (30% coarse maize; 6 mm soybean meal), treatment 7 (40% coarse maize; 6 mm soybean meal) and treatment 8 (50% coarse maize; 6 mm soybean meal). From day 7 to 14, treatment 2 which had the lowest percentage of coarse maize yielded the highest BW. This proves that birds younger than 14 days-of-age do not have sufficiently developed gizzards to handle more coarse maize in the diet. From day 21 to 35 the optimum inclusion rate of coarse maize was 30% although the difference at 28 and 35 days between the 30 and 50% coarse maize was not significant.

The treatments containing 4 mm soybean meal, treatment 3 (15% coarse maize; 4 mm soybean meal) and treatment 5 (30% coarse maize; 4 mm soybean meal), resulted in significant differences in the BW of broilers from day 7 to 35 with treatment 5 that contained 30% coarse maize yielding higher BW with the only exception being at 28 days-of-age. This further emphasises that 30% coarse maize in the diet may well be the optimum percentage of inclusion.

Kilbourn & Edwards (2001) found that reducing the particle size of the feed ingredients improves the nutrient utilisation by increasing the surface area exposed to digestive enzymes. Considering the results from treatment 4 (15% coarse maize; 2 mm soybean meal), treatment 10 (50% coarse maize; 2 mm soybean meal) and treatment 11 (100% fine maize; 2 mm soybean meal), this study found significant differences in the BW of broilers from day 7 to 35. Treatment 11 yielded the highest BW on day 7, 28 and 35, although on days 28 and 35 there was no significant difference between treatment 11 and treatment 4. Treatment 11 yielded the highest 35-day BW of all the treatments, thus suggesting that a smaller particle size does indeed present a bigger surface area to the digestive enzymes and improves nutrient utilisation by the bird to yield higher BW.

Table 4.1 Effect of maize and soybean meal (SBM) particle size on weekly bodyweight (g) of broilers (±standard error of the mean)

	Days-of-age					
Treatment	**0**	**7**	**14**	**21**	**28**	**35**
T1 - 15% coarse maize; unmilled SBM	44.67 (±1.32)	173.7^{A} (±7.26)	432.4^{A} (±15.9)	891.1^{A} (±23.68)	1495^{ABC} (±55.53)	2200^{BDC} (±68.32)
T12 - 50% coarse maize; unmilled SBM	43.95 (±0.95)	164.9^{DEF} (±4.37)	404.3^{F} (±10.39)	860.4^{C} (±16.19)	1497^{AB} (±30.28)	2170DE (±46.05)
T9 - 100% fine maize; unmilled SBM	44.22 (±1.19)	170.2^{ABC} (±6.38)	416.0 ^{BCDE} (±12.71)	855.2^{C} (±31.67)	1453^{C} (±69.83)	2187^{CD} (±58.18)
T2 - 15% coarse maize; 6mm SBM	44.32 (±1.52)	170.8^{ABC} (±4.70)	425.8^{AB} (±9.53)	852.0^{C} (±18.88)	1474^{BC} (±48.57)	2159DE (±38.48)
T3 - 15% coarse maize; 4mm SBM	43.52 (±1.45)	164.7^{DEF} (±8.48)	413.4^{CDEF} (±10.66)	868.5^{BC} (±11.58)	1504^{AB} (±27.04)	2190^{CD} (±30.69)
T4 - 15% coarse maize; 2mm SBM	44.23 (±1.40)	167.4^{CDE} (±6.86)	423.7^{AB} (±10.67)	900.4^{A} (±32.10)	1513^{AB} (±52.69)	2245^{A} (±50.02)
T2 - 15% coarse maize; 6mm SBM	44.32 (±1.52)	170.8^{ABC} (±4.70)	425.8^{AB} (±9.53)	852.0^{C} (±18.88)	1474^{BC} (±48.57)	2159DE (±38.48)
T6 - 30% coarse maize; 6mm SBM	43.72 (±1.85)	163.7^{EF} (±7.48)	411.5^{DEF} (±9.25)	869.4^{BC} (±33.04)	1512^{AB} (±55.37)	2226^{ABC} (±40.89)
T7 - 40% coarse maize; 6mm SBM	44.55 (±1.10)	157.5^{G} (±5.45)	404.4^{F} (±12.17)	860.4^{C} (±27.12)	1487^{ABC} (±37.74)	2196^{CD} (±46.83)
T8 - 50% coarse maize; 6mm SBM	44.48 (±0.87)	162.5^{F} (±5.45)	410.2^{EF} (±9.96)	862.1^{C} (±15.39)	1511^{AB} (±48.35)	2218^{ABC} (±50.61)
T3 - 15% coarse maize; 4mm SBM	43.52 (±1.45)	164.7^{DEF} (±8.48)	413.4^{CDEF} (±10.66)	868.5^{BC} (±11.58)	1504^{AB} (±27.04)	2190^{CD} (±30.69)
T5 - 30% coarse maize; 4mm SBM	44.37 (±1.24)	168.5^{BCD} (±6.23)	421.5^{BC} (±12.97)	884.6^{AB} (±31.12)	1484^{ABC} (±62.80)	2241^{AB} (±86.27)
T4 - 15% coarse maize; 2mm SBM	44.23 (±1.40)	167.4^{CDE} (±6.86)	423.7^{AB} (±10.67)	900.4^{A} (±32.10)	1513^{AB} (±52.69)	2245^{A} (±50.02)
T10 - 50% coarse maize; 2mm SBM	44.16 (±0.88)	161.6^{FG} (±3.689)	375.2^{G} (±9.86)	794.3^{D} (±30.39)	1391^{D} (±25.69)	2128^{E} (±41.70)
T11 - 100% fine maize; 2mm SBM	44.35 (±0.77)	172.4^{AB} (±4.72)	420.7^{BCD} (±12.80)	893.5^{A} (±38.18)	1521^{A} (±48.92)	2260^{A} (±33.28)

$^{A\text{-}G}$Means within a column without a common superscript are significantly different ($P < 0.05$)

4.2. The effect of maize and soybean meal particle size on broiler feed intakes

Table 4.2 shows the cumulative feed intake (FI) per bird from day 0 to 35. The results from this study shows that only at day 14 there were significant differences in the FI between the treatments. Considering treatment 1 (15% coarse maize; unmilled soybean meal), treatment 12 (50% coarse maize; unmilled soybean meal) and treatment 9 (100% fine maize; unmilled soybean meal) it can be concluded that birds of 7 – 14 days-of-age ate more of treatment 1 and treatment 9 which contained more fine maize than T12. This may be due to the fact that their digestive tracts had not yet fully developed and that they struggled to digest the feed when it contained higher percentages of coarse maize. Larger particles will be retained for longer periods of time than finer particles in the digestive tract (Denbow, 2000), prolonging mean residence time.

The treatments containing more coarse maize will thus have a slower passage rate and according to Svihus *et al.* (2002), slow passage rate limits the intake of feed. When comparing treatment 2 (15% coarse maize; 6 mm soybean meal), treatment 3 (15% coarse maize; 4 mm soybean meal) and treatment 4 (15% coarse maize; 2 mm soybean meal) at 14 days-of-age, it can clearly be seen that the birds did eat significantly less as the particle size of the soybean meal decreased, which perhaps were due to the fact that they were getting sufficient nutrients from less feed as the surface area of the digesta increase (Kilburn & Edwards, 2001). The birds on these three different treatments (2, 3 & 4) had lower FI from day 0 to 35 as the soybean meal particle size decreased, although there was no significant difference in the cumulative FI at day 35.

Significant differences were also found between treatments 2 (15% coarse maize), 6 (30% coarse maize), 7 (40% coarse maize) and 8 (50% coarse maize) which all contained 6 mm soybean meal at day 14. There was however no clear pattern. The FI was the highest for T2 from day 0 to 35. Birds fed treatment 3 (15% coarse maize; 4 mm soybean meal) had a higher FI than the birds fed treatment 5 (30% coarse maize; 4 mm soybean meal), although only being significantly different for the period between day 7 and 14. This may also be due to the longer retention time of more coarse particles in T5. Treatments containing 2 mm soybean meal, 4 (15% coarse maize), 10 (50% coarse maize) and 11 (100% fine maize) showed the same pattern where the FI reduced as the amount of coarse maize in die diet fed increased, also only being significantly different for the period of day 7 to 14.

Table 4.2 Effect of maize and soybean meal (SBM) particle size on weekly feed intake (g) of broilers (± standard error of the mean)

	Days-of-age				
Treatment	**7**	**14**	**21**	**28**	**35**
T1 - 15% coarse maize; unmilled SBM	158.7 (±13.98)	541.7^{ABC} (±16.56)	1167 (±39.72)	2112 (±68.79)	3166 (±108.19)
T12 - 50% coarse maize; unmilled SBM	159.3 (±12.88)	527.2^{CD} (±12.39)	1146 (±24.25)	2078 (±41.92)	3131 (±57.79)
T9 - 100% fine maize; unmilled SBM	161.6 (±12.79)	539.6^{ABC} (±14.62)	1160 (±21.59)	2109 (±30.50)	3180 (±57.64)
T2 - 15% coarse maize; 6mm SBM	168.7 (±12.51)	548.2^{A} (±16.32)	1178 (±29.47)	2152 (±44.69)	3216 (±64.48)
T3 - 15% coarse maize; 4mm SBM	161.5 (±12.05)	540.7^{ABC} (±17.75)	1176 (±31.00)	2128 (±51.20)	3206 (±83.68)
T4 - 15% coarse maize; 2mm SBM	157.0 (±7.93)	535.6^{ABCD} (±22.65)	1169 (±35.39)	2113 (±49.29)	3160 (±87.34)
T2 - 15% coarse maize; 6mm SBM	168.7 (±12.51)	548.2^{A} (±16.32)	1178 (±29.47)	2152 (±44.69)	3216 (±64.48)
T6 - 30% coarse maize; 6mm SBM	156.4 (±9.22)	524.3^{D} (±11.34)	1157 (±56.73)	2111 (±93.75)	3176 (±126.53)
T7 - 40% coarse maize; 6mm SBM	156.6 (12.66)	530.2^{CD} (±14.64)	1156 (±40.13)	2106 (±75.55)	3157 (±101.87)
T8 - 50% coarse maize; 6mm SBM	167.0 (±16.42)	532.4^{BCD} (18.67)	1155 (±24.76)	2106 (±48.40)	3169 (±75.78)
T3 - 15% coarse maize; 4mm SBM	161.5 (±12.05)	540.7^{ABC} (±17.75)	1176 (±31.00	2128 (±51.20)	3206 (±83.68)
T5 - 30% coarse maize; 4mm SBM	167.8 (±20.13)	534.6^{ABCD} (±22.03)	1152 (±55.98)	2110 (±70.02)	3167 (±96.20)
T4 - 15% coarse maize; 2mm SBM	157.0 (±7.93)	535.6^{ABCD} (±22.65)	1169 (±35.39)	2113 (±49.29)	3160 (±87.34)
T10 - 50% coarse maize; 2mm SBM	168.1 (±9.77)	534.2^{ABCD} (±19.49)	1143 (±54.51)	2086 (±85.04)	3126 (±109.58)
T11 - 100% fine maize; 2mm SBM	166.6 (±6,47)	546.8^{AB} (±16.69)	1175 (±32.67)	2136 (±50.02)	3217 (±71.77)

$^{A\text{-}D}$Means within a column without a common superscript are significantly different ($P < 0.05$)

4.3. The effect of maize and soybean meal particle size on feed conversion ratio

Rougière *et al.* (2009) found that feed efficiency was lower in birds fed fine particles compared to birds fed coarse particles. Xu *et al.* (2017) found that the replacement of 20% of fine maize with coarse maize had no effect on broiler performance from day 0 to 14, but when the coarse maize inclusion level was 50%, feed conversion ratio (FCR) and weight gain were consistently improved after 14 days of age. Xu *et al*. (2015) reported that replacing 50% fine maize with 50% coarse maize in a pelleted broiler diet reduced FCR from day 0 to 35 and day 0 to 49 and increased weight gain from day 0 to 35.

Table 4.3 shows the FCR of the birds from day 0 to 35. In this study, there was no significant difference between the treatments for FCR on day 7. This might be due to the fact that the digestive tracts of the birds are not developed sufficiently during the first couple of days. There were significant differences in the FCR of treatment 1 (15% coarse maize; unmilled soybean meal), treatment 12 (50% coarse maize; unmilled soybean meal) and treatment 9 (100% fine maize; unmilled soybean meal) from day 14 to 35. In contrast to the findings of Rougière *et al.* (2009), treatment 9 that contained 100% fine maize had the highest FCR and treatment 1 had the lowest FCR.

Treatments 2, 3 and 4 paints a different picture in terms of soybean meal particle size. There were statistical significant differences between treatment 2 (15% coarse maize; 6 mm soybean meal), treatment 3 (15% coarse maize; 4 mm soybean meal) and treatment 4 (15% coarse maize; 2 mm soybean meal) from day 14 to 35. From day 21 to 35, when the birds start putting on major amounts of especially breast muscle, the FCR reduced as the soybean meal particle size in the diet decreased. Between the treatments that contained 6 mm soybean meal, treatment 6 with 30% coarse maize had a lower FCR than treatments 2 (15% coarse maize), 7 (40% coarse maize) and 8 (50% coarse maize). With the exception of day 28, treatment 6 had a significantly lower FCR than treatment 2, 7 and 8, but no clear pattern could be concluded between these treatments. When comparing treatment 3 (15% coarse maize; 4 mm soybean meal) and treatment 5 (30% coarse maize; 4 mm soybean meal), with the exception of day 28, treatment 5 that contained 30% coarse maize had a significantly lower FCR than treatment 3. From what was seen by comparing treatments 10 and 11, the findings of Rougière *et al.* (2009) does hold true when relatively fine soybean meal is included in the diet.

Treatment 10 (2 mm soybean meal), which had 50% coarse maize, did give a significantly higher FCR value from day 14 to 35 than treatment 11 (100% fine maize; 2 mm soybean meal). But then, in contrast to this, treatment 4 (15% coarse maize; 2 mm soybean meal) did give rise to significantly lower FCR value from day 14 to 35 when compared to treatment 11.

Table 4.3 Effect of maize and soybean meal (SBM) particle size on the cumulative feed conversion ratio of broilers (± standard error of the mean)

	Days-of-age				
Treatment	**0 - 7**	**0 - 14**	**0 - 21**	**0 - 28**	**0 - 35**
T1 - 15% coarse maize; unmilled SBM	1.265 (±0.131)	1.398^{F} (±0,040)	1.379^{EFG} (±0.049)	1.457^{CD} (±0.045)	1.469^{BCDEF} (±0.038)
T12 - 50% coarse maize; unmilled SBM	1.316 (±0.097)	1.464^{BC} (±0.049)	1.404^{CDEFG} (±0.042)	1.431^{D} (±0.030)	1.473^{BCDE} (±0.039)
T9 - 100% fine maize; unmilled SBM	1.286 (±0.124)	1.450^{BCDE} (±0.037)	1.432^{BC} (±0.038)	1.501^{BC} (±0.067)	1.485^{ABCD} (±0.032)
T2 - 15% coarse maize; 6mm SBM	1.336 (±0.115)	1.438^{BCDEF} (±0.048)	1.459^{B} (±0.040)	1.506^{AB} (±0.044)	1.521^{A} (±0.025)
T3 - 15% coarse maize; 4mm SBM	1.339 (±0.146)	1.462^{BC} (±0.042)	1.426^{BDC} (±0.028)	1.457^{CD} (±0.044)	1.494^{ABC} (±0.035)
T4 - 15% coarse maize; 2mm SBM	1.279 (±0.118)	1.411^{EF} (±0.052)	1.376^{G} (±0.050)	1.439^{D} (±0.032)	1.436^{F} (±0.037)
T2 - 15% coarse maize; 6mm SBM	1.336 (±0.115)	1.438^{BCDEF} (±0.048)	1.459^{B} (±0.040)	1.506^{AB} (±0.044)	1.521^{A} (±0.025)
T6 - 30% coarse maize; 6mm SBM	1.309 (±0.124)	1.427^{CDEF} (±0.043)	1.402^{CDEFG} (±0.054)	1.438^{D} (±0.019)	1.455^{DEF} (±0.052)
T7 - 40% coarse maize; 6mm SBM	1.388 (±0.119)	1.474^{B} (±0.038)	1.417^{BCDE} (±0.049)	1.460^{CD} (±0.054)	1.468^{BCDEF} (±0.039)
T8 - 50% coarse maize; 6mm SBM	1.415 (±0.124)	1.456^{BCD} (±0.031)	1.413^{CDEF} (±0.033)	1.437^{D} (±0.053)	1.458^{CDEF} (±0.029)
T3 - 15% coarse maize; 4mm SBM	1.339 (±0.146)	1.462^{BC} (±0.042)	1.426^{BDC} (±0.028)	1.457^{CD} (±0.044)	1.494^{ABC} (±0.035)
T5 - 30% coarse maize; 4mm SBM	1.355 (±0.190)	1.418^{DEF} (0.045)	1.372^{FG} (±0.050)	1.467^{BCD} (±0.065)	1.443^{EF} (±0.054)
T4 - 15% coarse maize; 2mm SBM	1.279 (±0.118)	1.411^{EF} (±0.052)	1.3766^{G} (±0.050)	1.439^{D} (±0.032)	1.436^{F} (±0.037)
T10 - 50% coarse maize; 2mm SBM	1.43 (±0.099)	1.615^{A} (±0.076)	1.526^{A} (±0.091)	1.549^{A} (±0.060)	1.500^{AB} (±0.056)
T11 - 100% fine maize; 2mm SBM	1.343 (±0.141)	1.453^{BCD} (±0.028)	1.385^{DEFG} (±0.045)	1.448^{D} (±0.051)	1.452^{DEF} (±0.023)

$^{A\text{-}G}$Means within a column without a common superscript are significantly different ($P < 0.05$)

4.4. The effect of maize and soybean meal particle size on broiler production efficiency factor

To compare broiler results between the different treatments the production efficiency factor (PEF) can be used. This factor standardises technical results, taking into account the FCR, mortalities and daily gain.

Table 4.4 shows the PEF values for the different treatments. This study found significant different PEF values for treatment 1 (15% coarse maize; unmilled soybean meal), treatment 12 (50% coarse maize; unmilled soybean meal) and treatment 9 (100% fine maize; unmilled soybean meal) with treatment 1 giving the best overall broiler performance between these 3 treatments, showing that when unmilled soybean meal is included in the diet, 15% coarse maize performed the best. This higher PEF value of treatment 1 is due to the heavier birds and lower cumulative FCR when compared to treatments 9 and 12. There were significant differences in PEF values between treatment 2 (15% coarse maize; 6 mm soybean meal), treatment 3 (15% coarse maize; 4 mm soybean meal) and treatment 4 (15% coarse maize; 2 mm soybean meal). The PEF value increased as the particle size of the soybean meal in the diet was reduced, with treatment 4 giving the highest value. Treatment 4 also resulted in the heaviest birds (2245 g at 35 days-of-age) and lowest cumulative FCR (1.436), thus yielding the highest PEF value.

Between treatments 2 (15% coarse maize; 6 mm soybean meal), 6 (30% coarse maize; 6 mm soybean meal), 7 (40% coarse maize; 6 mm soybean meal), and 8 (50% coarse maize; 6 mm soybean meal), there were significant differences in PEF values, but no pattern. When soybean meal milled on a roller mill with a screen size of 6 mm was included in the diet, treatment 6 and 8, which contained 30% and 50% coarse maize respectively, performed the best. When the diet contained soybean meal milled on a roller mill with a screen size of 4 mm (treatment 3 and 5), treatment 5 containing 30% coarse maize outperformed treatment 3 containing only 15% coarse maize. Treatment 3 yielded heavier birds (2241g at 35 days-of-age) and had a lower FCR (1.44) which causes the higher PEF value. Lastly, when a diet contains soybean meal milled on a roller mill with a screen size of 2 mm (treatments 4, 10 & 11), T4 containing 15% coarse maize performed the best. Treatment 4 also performed the best overall in terms of PEF value. Treatment 4 yielded the second heaviest birds at 35 days-of-age and the lowest FCR of all the treatments, which contributed to having the highest PEF value.

Table 4.4 Effect of maize and soybean meal (SBM) particle size on the production efficiency factor (PEF) of broilers at 35 days-of-age (±standard error of the mean)

Treatment	PEF
T1 - 15% coarse maize; unmilled SBM	416^{BDC} (±22.04)
T12 - 50% coarse maize; unmilled SBM	412^{CDEF} (±19.93)
T9 - 100% fine maize; unmilled SBM	415^{BCDE} (±16.25)
T2 - 15% coarse maize; 6mm SBM	398^{F} (±10.54)
T3 - 15% coarse maize; 4mm SBM	408^{DEF} (±16.54)
T4 - 15% coarse maize; 2mm SBM	435^{A} (±16.58)
T2 - 15% coarse maize; 6mm SBM	398^{F} (±10.54)
T6 - 30% coarse maize; 6mm SBM	429^{ABC} (±17.51)
T7 - 40% coarse maize; 6mm SBM	421^{ABCD} (±20.41)
T8 - 50% coarse maize; 6mm SBM	430^{AB} (±14.10)
T3 - 15% coarse maize; 4mm SBM	408^{DEF} (±16.54)
T5 - 30% coarse maize; 4mm SBM	434^{A} (±32.18)
T4 - 15% coarse maize; 2mm SBM	435^{A} (±16.58)
T10 - 50% coarse maize; 2mm SBM	398^{EF} (±17.15)
T11 - 100% fine maize; 2mm SBM	433^{A} (±12.37)

$^{A-F}$Means within a column without a common superscript are significantly different ($P < 0.05$)

4.5. The effect of maize and soybean meal particle size on gizzard and proventriculus development

The particle size of feed ingredients influence the development of the digestive tract, especially the gizzard and proventriculus of broilers. Nir *et al.* (1994) found greater gizzard development and lowered pH in the gizzard of broilers fed diets which contained coarse particles compared to diets containing only fine particles.

The main function of the gizzard is the fine grinding of the ingested foods and when this function is carried out by the feed mill, it has a detrimental effect on gizzard size and function (Duke, 1986). This then results in a gizzard that is underdeveloped and an enlarged proventriculus (Taylor & Jones, 2004). Peron *et al.* (2005) found that pelleted diets that contained coarse particles significantly increased gizzard weights when compared to finer particle diets.

Amerah *et al.* (2018) found no significant effect of diet particle size on the tissue weight of the gut components relative to the BW, including the gizzard and proventriculus. They specifically found no significant difference in the relative weights (g/kg of BW) of the gizzard and proventriculus of birds fed medium and coarse particle size pelleted wheat based diets.

Table 4.5 shows the gizzard and proventriculus weights as a percentage of the total BW of the bird found in the present study. Results in terms of gizzard and proventriculus development were not conclusive. Some significant differences in the gizzard weights as a percentage of the total BW at day 35 were found, but there was no real and significant pattern.

Considering treatment 1 (15% coarse maize; unmilled soybean meal), treatment 12 (50% coarse maize; unmilled soybean meal) and treatment 9 (100% fine maize; unmilled soybean meal), it can be seen that treatment 12 that contained the highest percentage of coarse particles resulted in the highest gizzard as a % of the BW which agrees with the findings of Peron *et al.* (2005). This can also be seen between treatment 3 (15% coarse maize; 4 mm soybean meal) and treatment 5 (30% coarse maize; 4 mm soybean meal), where treatment 5 had a significantly higher gizzard to BW ratio than treatment 3. This study found no significant difference in the proventriculus to BW ratio between the different treatments.

Considering treatment 1 (15% coarse maize; unmilled soybean meal), treatment 12 (50% coarse maize; unmilled soybean meal) and treatment 9 (100% fine maize; unmilled soybean meal), the biggest gizzard as a % of the bird's BW was found in treatment 12 which also contained the most coarse maize. However, treatment 12 had the lowest PEF value (412) of the three treatments. Treatment 2 (15% coarse maize; 6 mm soybean meal), treatment 3 (15% coarse maize; 4 mm soybean meal) and treatment 4 (15% coarse maize; 2 mm soybean meal), showed the same pattern. Treatment 2 contained the most coarse particles and had the highest gizzard as a % of the bird's BW value, but had the lowest PEF value (398) of the three. Treatment 4 (15% coarse maize; 2 mm soybean meal), treatment 10 (50% coarse maize; 2 mm soybean meal) and treatment 11 (100% fine maize; 2 mm soybean meal), also showed this pattern. Treatment 10 contained the most coarse particles and had the highest gizzard as a % of the bird's BW value but the lowest PEF (398) value of the three. This would suggest that having a big and well-developed gizzard is not as important as feeding the correct particle size for maximum broiler performance. Treatment 11 contained 100% fine maize and 2 mm soybean meal, but still had a relatively well-developed gizzard, which agrees with the findings of Amerah *et al.* (2018) and yielded a very good PEF value of 433.

Table 4.5 Effect of maize and soybean meal (SBM) particle size on gizzard and proventriculus weight as a % of bodyweight (± standard error of the mean)

	7 days-of-age		14 days-of-age		35 days-of-age	
Treatment	**Gizzard**	**Proventriculus**	**Gizzard**	**Proventriculus**	**Gizzard**	**Proventriculus**
T1 - 15% coarse maize; unmilled SBM	3.33 (±0.74)	0.75 (±0.14)	2.82 (±0.51)	0.61 (±0.07)	1.56^{BCD} (±0.14)	0.38 (±0.05)
T12 - 50% coarse maize; unmilled SBM	3.53 (±0.64)	0.79 (±0.17)	2.81 (±0.27)	0.62 (±0.06)	1.69^{AB} (±0.23)	0.39 (±0.04)
T9 - 100% fine maize; unmilled SBM	3.13 (±0.35)	0.75 (±0.09)	2.87 (±0.17)	0.66 (±0.06)	1.60^{ABC} (±0.18)	0.38 (±0.05)
T2 - 15% coarse maize; 6mm SBM	3.07 (±0.48)	0.75 (±0.16)	2.88 (±0.30)	0.64 (±0.06)	1.67^{AB} (±0.27)	0.39 (±0.06)
T3 - 15% coarse maize; 4mm SBM	3.29 (±0.55)	0.79 (±0.15)	2.92 (±0.44)	0.65 (±0.04)	1.41^{D} (±0.18)	0.34 (±0.06)
T4 - 15% coarse maize; 2mm SBM	3.29 (±0.62)	0.72 (±0.19)	2.83 (±0.32)	0.62 (±0.09)	1.47^{CD} (±0.13)	0.40 (0.06)
T2 - 15% coarse maize; 6mm SBM	3.07 (±0.48)	0.75 (±0.16)	2.88 (±0.30)	0.64 (±0.06)	1.67^{AB} (±0.27)	0.39 (±0.06)
T6 - 30% coarse maize; 6mm SBM	3.15 (±0.47)	0.76 (±0.16)	2.77 (±0.26)	0.64 (±0.07)	1.57^{BCD} (±0.23)	0.37 (±0.04)
T7 - 40% coarse maize; 6mm SBM	3.38 (±0.58)	0.76 (±0.17)	2.97 (±0.35)	0.62 (±0.05)	1.76^{A} (±0.14)	0.40 (±0.04)
T8 - 50% coarse maize; 6mm SBM	3.27 (±0.61)	0.78 (±0.15)	2.80 (±0.24)	0.66 (±0.06)	1.57^{BCD} (±0.13)	0.38 (±0.06)
T3 - 15% coarse maize; 4mm SBM	3.29 (±0.55)	0.79 (±0.15)	2.92 (±0.44)	0.65 (±0.04)	1.41^{D} (±0.18)	0.34 (±0.06)
T5 - 30% coarse maize; 4mm SBM	3.35 (±0.66)	0.8 (±0.20)	2.85 (±0.35)	0.60 (±0.07)	1.52^{BCD} (±0.16)	0.36 (±0.05)
T4 - 15% coarse maize; 2mm SBM	3.29 (±0.62)	0.72 (±0.19)	2.83 (±0.32)	0.62 (±0.09)	1.47^{CD} (±0.13)	0.40 (0.06)
T10 - 50% coarse maize; 2mm SBM	3.35 (±0.58)	0.75 (±0.13)	2.88 (±0.39)	0.66 (±0.06)	1.63^{ABC} (±0.27)	0.39 (±0.04)
T11 - 100% fine maize; 2mm SBM	3.26 (±0.27)	0.76 (±0.14)	2.93 (±0.27)	0.64 (±0.06)	1.60^{ABC} (±0.23)	0.39 (±0.07)

$^{A\text{-}D}$Means within a column without a common superscript are significantly different ($P < 0.05$)

4.6 The effect of maize and soybean meal particle size on pellet hardness and thus durability

Feeding broilers on a pelleted diet holds many advantages like higher feed intake, better feed efficiency and better overall performance, to name a few. Good pellet quality is important as it has to go through bagging, transport, handling and storage without breaking up and turning into fines before it reaches the feeders (Amerah *et al*., 2007a).

Having a good pellet means that pellets will more likely remain intact until the time of feeding. Carre *et al.* (2005) found a positive correlation between pellet durability and feed efficiency. Pellet durability is inversely related to particle size (Angulo *et al*., 1996). The smaller particles have more contact points with each other because of their larger surface area (Behnke, 1996), thus meaning that smaller particles in pelleted diets should result in better pellet durability. Svihus *et al*. (2004) suggested that a high inclusion level of coarse particles in the diet will result in pellets with a lower durability compared to pellets made with fine particles.

Table 4.6 shows the pellet hardness of the grower and finisher phase diets for the various treatments. This study found significant differences between the pellet hardness of the different treatments for the grower and finisher phase diets. Considering treatments 1 (15% coarse maize; unmilled soybean meal), 12 (50% coarse maize; unmilled soybean meal) and 9 (100% fine maize; unmilled soybean meal), it can be concluded that treatment 1 than contained 15% coarse maize had the highest pellet hardness of the three. Treatment 12 containing 50% coarse maize had the lowest pellet hardness and would have had a low pellet durability, which agrees with the findings of Svihus *et al*. (2004). Between treatments 2 (15% coarse maize; 6 mm soybean meal), 3 (15% coarse maize; 4 mm soybean meal) and 4 (15% coarse maize; 2 mm soybean meal), treatment 2 yielded the lowest pellet hardness and treatment 4 yielded the highest. This also showed a clear pattern in that the pellet hardness increased as the soybean meal particle size decreased and it can also be concluded that treatment 4 with the hardest pellets, resulted in the best feed conversion ratio. Treatments 2 (15% coarse maize; 6 mm soybean meal), 6 (30% coarse maize; 6 mm soybean meal), 7 (40% coarse maize; 6 mm soybean meal) and 8 (50% coarse maize; 6 mm soybean meal) did not show such a clear pattern but it can still be concluded that treatment 8, that contained 50% coarse maize, yielded very low pellet hardness and thus pellet durability. Treatment 3 (15% coarse maize; 4 mm soybean meal) and treatment 5 (30% coarse maize; 4 mm soybean meal) showed that treatment 3 with only 15% coarse maize had a higher pellet hardness than treatment 5 with 30%.

When examining the results of treatments 4 (15% coarse maize; 2 mm soybean meal), 10 (50% coarse maize; 2 mm soybean meal) and 11 (100% fine maize; 2 mm soybean meal) it can be concluded that some coarse maize particles (treatment 4) yielded a significantly higher pellet hardness than the high level of coarse maize (treatment 10) or no coarse maize at all (treatment 11). Treatment 4 with a harder pellet also resulted in the lowest feed conversion ratio of the three treatments. Overall it was concluded that for a good pellet hardness and pellet durability some coarse particles are needed in the pellet, but not too much. This may be due to the fact that some coarse particles may provide structural support to help keep all the other fine particles together.

Table 4.6 Effect of maize and soybean meal (SBM) particle size on the pellet hardness (kg) of the grower and finisher phases of the diet (± standard error of the mean)

Treatment	Grower	Finisher
T1 - 15% coarse maize; unmilled SBM	5.33^{B} (± 0.95)	5.17^{DE} (± 0.83)
T12 - 50% coarse maize; unmilled SBM	2.67^{F} (± 0.61)	2.19^{H} (± 0.62)
T9 - 100% fine maize; unmilled SBM	3.56^{DE} (± 0.41)	4.98^{E} (± 0.62)
T2 - 15% coarse maize; 6mm SBM	3.41^{E} (± 0.85)	5.41^{CD} (± 0.83)
T3 - 15% coarse maize; 4mm SBM	3.90^{D} (± 0.65)	5.56^{C} (± 0.81)
T4 - 15% coarse maize; 2mm SBM	6.43^{A} (± 0.99)	6.99^{A} (± 0.94)
T2 - 15% coarse maize; 6mm SBM	3.41^{E} (± 0.85)	5.41^{CD} (± 0.83)
T6 - 30% coarse maize; 6mm SBM	3.68^{DE} (± 0.78)	3.12^{F} (± 0.63)
T7 - 40% coarse maize; 6mm SBM	3.50^{E} (± 0.55)	2.60^{G} (± 0.79)
T8 - 50% coarse maize; 6mm SBM	2.78^{F} (± 0.40)	2.12^{H} (± 0.59)
T3 - 15% coarse maize; 4mm SBM	3.90^{D} (± 0.65)	5.56^{C} (± 0.81)
T5 - 30% coarse maize; 4mm SBM	3.79^{DE} (± 0.85)	5.23^{CDE} (± 0.79)
T4 - 15% coarse maize; 2mm SBM	6.43^{A} (± 0.99)	6.99^{A} (± 0.94)
T10 - 50% coarse maize; 2mm SBM	4.78C (± 0.77)	5.54^{CD} (± 0.65)
T11 - 100% fine maize; 2mm SBM	5.36^{B} (± 0.81)	6.60^{B} (± 0.79)

$^{A-F}$Means within a column without a common superscript are significantly different ($P < 0.01$)

Chapter 5

Conclusion

Decreasing the particle size of maize and/or soybean meal improved performance in Ross 308 broilers, thus confirming the first alternative hypothesis. From the results of this study it can be concluded that maize and soybean particle size in a pelleted diet had a significant effect on overall broiler performance. The broiler performance increased significantly as the particle size of the soybean meal in the pelleted diet decreased from 6 mm (PEF of 398) to 2 mm (PEF of 435) with a 15% coarse maize inclusion level. This was due to higher bird weights at 35 days-of-age and better (lower) cumulative FCR values. The higher bird weights and lower FCR values may be due to the fact that the smaller soybean meal particles are more digestible because of a bigger surface area that gets exposed in the GIT of the bird, thus resulting in better absorption into the body.

For optimum broiler performance some coarse maize (15%) and not just fine maize is needed in the pelleted diet. There was however, no significant difference between the PEF value for treatment 4 (15% coarse maize; 2 mm soybean meal) and treatment 11 (100% fine maize; 2 mm soybean meal). It can thus be concluded that there is no need for coarse maize in a pelleted diet fed to broilers when there is 2 mm soybean meal in the diet.

Maize and soybean meal particle size did not have a significant effect on gizzard and proventriculus development in Ross 308 broilers during the first two weeks. Gizzard development was significantly improved by the presence of coarser materials in the diet, regardless whether it was from the maize or from the soybean meal fraction. The second alternative hypothesis can therefore be accepted, as it was evident that particle size affected gizzard development. However, the treatments that yielded better developed gizzards did not yield better broiler production performance (PEF values). No effect was seen on the proventriculus development throughout the production cycle.

Decreasing maize and soybean meal particles had a positive effect on pellet hardness. Pellet hardness and thus pellet durability was influenced by the maize and soybean meal particle size in the mash prior to pelleting. The pellet hardness and pellet durability increased as the soybean meal particle size decreased thus confirming the third alternative hypothesis. For optimum pellet hardness and better pellet durability the mash must contain some coarse maize particles prior to pelleting to provide structural support, but no more than 15%. The treatments with better pellet hardness and pellet durability resulted in better feed conversion ratios, which emphasises that pellet hardness is really important in order to achieve a high level of production performance.

During this study, different combinations of maize and soybean meal particle size affected bird performance. Treatment 4 which contained 15% coarse maize and 2 mm soybean meal yielded the best broiler performance (PEF of 435) and treatment 10 which contained 50% coarse maize and 2 mm soybean meal yielded the worse broiler performance (PEF of 398). There were significant differences in the overall broiler performance between most of the treatments. For optimum broiler performance a pelleted diet with a 15% coarse maize and 2 mm soybean meal is recommended.

It can be concluded that the soybean meal in broiler diets should not be used as received from the suppliers as it is customary at the moment. It should rather be milled through a 2 mm screen size when used as a constituent in broiler feed. This will result in higher bodyweights, better FCR and thus better PEF values. The improved efficiency will save on resources to produce food for human consumption.

Overall from this study it can be concluded that soybean meal and maize particle size can be a valuable tool and means of improving broiler production, to improve the efficiency of the utilisation of our natural resources and ensure that we are able to supply in the global demand for food of animal origin in the years to come.

Critical review and recommendations

1. Further research is needed to establish the optimum soybean meal particle size. This study included four different particle sizes, a further study with more treatments with different particle sizes will be of value.
2. To accurately define the particle size of the maize and soybean meal, both have to be sieved on different screen sizes after milling to ensure that each treatment have definite particle sizes. In this study, it was roller-milled through different screen sizes. For example: If you sieve the soybean meal through a 2 mm after milling, all the particles should be smaller than 2 mm.
3. This study showed that some coarse maize and not only fine maize is needed in the diet to obtain the best performance. Further research is needed to define exactly how much of the total fraction of maize must be coarse.
4. Extended research is required to accurately quantify the effects of feed particle size on gizzard and proventriculus development. The sample size from which the gizzard and proventriculus were taken was relatively small and this small sample may have influenced the overall results observed. A larger sample size may have provided more conclusive results.

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Appendix

Appendix A Temperature profile of the trial house from 2 days before placement to slaughtering at 35 days

Day	Target floor temperature (°C, 50 % RH[1])
1 day before placement to 2	35.5
3 to 5	34.5
6 to 8	33.5
9 to 11	29.7
12 to 14	27.2
15 to 17	26.2
18 to 20	25.0
21 to 23	24.0
24 to 35	23.0

[1]RH=Relative Humidity

Appendix B Lighting program of the trial house from placement of the Ross broiler chicks to slaughter at 35 days-of-age

Day	Controller's set point			
	Lights on	Lights off	Hours of Daylight	Hours of Darkness
1 to 3	00:00	23:00	23	1
4 to 8	00:00	21:00	21	3
9 to 11	05:00	22:00	17	7
12 to 15	05:00	20:00	15	9
16 to 33	05:00	19:00	14	10
34 to	02:00	22:00	20	4

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Appendix C Vaccination program (New Castle Disease and Infectious Bronchitis) of the Ross 308 broilers during the trial

Age (days)	Vaccination	Method	Trade name	Supplier
Hatchery	NCB[1]	Spray	Avinew	Merial South Africa (Pty) Ltd
Hatchery	IB[2]	Spray	Bioral H120	Merial South Africa (Pty) Ltd
10-12 days	NCB	Water	TAbic VH	Phibro Animal Health
10-12 days	IB	Water	TAbic MB	Phibro Animal Health
16-18 days	NCB	Water	Avinew	Merial South Africa (Pty) Ltd

[1] NCB = New Castle Disease
[2] IB = Infectious Bronchitis

Appendix D Layout of the pens and blocks in the trial house with the random treatment allocations to each pen

House A						House B			
Pen	Treat	Treat	Pen			Pen	Treat	Treat	Pen
1	K	F	64	1	1	64	B	I	1
2	A	I	63			63	A	K	2
3	H	E	62			62	D	L	3
4	B	C	61			61	F	J	4
5	G	D	60			60	G	H	5
6	J	L	59			59	E	C	6
7	E	A	58	2	2	58	L	A	7
8	I	G	57			57	H	F	8
9	J	B	56			56	I	C	9
10	H	C	55			55	J	D	10
11	K	D	54			54	K	G	11
12	L	F	53			53	B	E	12
13	J	H	52	3	3	52	G	I	13
14	I	E	51			51	C	A	14
15	D	A	50			50	H	B	15
16	C	F	49			49	K	L	16
17	L	B	48			48	D	F	17
18	K	G	47			47	E	J	18
19	J	K	46	4	4	46	D	E	19
20	I	H	45			45	A	C	20
21	A	G	44			44	H	I	21
22	L	C	43			43	L	B	22
23	B	D	42			42	G	K	23
24	E	F	41			41	J	F	24
25	K	I	40	5	5	40	H	D	25
26	J	L	39			39	C	F	26
27	D	G	38			38	B	K	27
28	F	C	37			37	J	G	28
29	H	E	36			36	L	E	29
30	B	A	35			35	A	I	30
31			34			34			31
32			33			33			32

Appendix E The feeding phases with feeding periods and expected intakes per bird.

Feed (5 phases)	**Feeding period (days)**	**Feed intake (g/bird)**	**Feed allocation/pen (kg)**
Starter	17	1030	62
Grower	10	1417	85
Finisher	8	1663	98