The inclusion of a *Bacillus*-based probiotic in feed to increase the dietary energy available to growing pigs

by

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DECLARATION

I, **Etienne Biddulph** the undersigned, declare that this dissertation/thesis, which I hereby submit for the degree M.Sc. Animal Science: Animal Production at the University of Pretoria, is my own work and has not previously been submitted by me or another individual for a degree at this or any other tertiary institution.

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E. Biddulph

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ABSTRACT

The livestock industry has come under increasing scrutiny for using antibiotics sub-therapeutically as growth promoters. Increased awareness of the negative effects of microbial antibiotic resistance has amplified consumer pressure to raise livestock without the use of these growth promoters. Feed additives and more specifically probiotics could act as an alternative to sub therapeutic AGP use. The aim of this study was to evaluate the effects of a spore-forming probiotic (Bioplus YC, Chr. Hansen Denmark) on the nutrient availability of pig feeds and whether the specific probiotic is able to release additional energy (0.3 MJ/kg or 72 kcal/kg on net energy level) from the undigested or unabsorbed dietary fibre fraction. To achieve the aim, the performance of pigs receiving standard and reduced energy diets, with and without a commercially available probiotic feed additive, was measured.

A completely randomised block design experiment was conducted at the University of Pretoria's experimental farm. One hundred and seventy four male pigs from the PIC 337 line (Pig Improvement Company, USA) with an average body weight of 6.81 kg ± 0.587 kg, were obtained from a local commercial farm. The piglets were randomly selected on weaning day from that week's weaned piglet batch on 21-days of age. Pigs were randomly allotted to 1 of 4 treatments in a 2x2 factorial arrangement of treatments. Five feeding phases were used to feed 168 male pigs over an 18-week (126 days) trial period. Four treatments were fed during each phase which included two standard energy diets (Treatment 1 and 2) and two reduced energy diets (Treatment 3 and 4). The lower energy diets (Treatment 3 and Treatment 4) were reduced by 0.3 MJ/kg or 72 kcal/kg on net energy level (NE). Treatment 1 and 3 was supplemented with the probiotic at a manufacturer's standard dosage of 400 mg/kg of final feed. Production parameters (body weight, average daily gain, feed intake, feed conversion ratio and faecal scoring) were measured weekly and per dietary phase. Slaughter parameters (carcass weight, backfat thickness and lean meat percentage) were measured during the slaughter of the pigs at day 148 of age.

Supplementing commercial pig diets with a dual strain probiotic significantly improved body weight and body weight gain from the grower 1 phase onwards until slaughter without affecting the feed intake of animals. The addition of a probiotic could influence the cumulated FCR of supplemented pigs in reduced energy diets when compared to standard energy diets. Positive effects were noted in the carcass weight of probiotic supplemented vs. non supplemented animals. Probiotic supplemention of a reduced energy diet resulted in significantly larger carcasses when compared to the unsupplemented reduced energy group. The compounding effects of a beneficial microbiota balance from weaning, together with the various modes of action that the probiotic enables on the GIT over the entire growing period, most possibly contributed to the positive results seen on production parameters.The probiotic used in this study showed potential as a viable alternative to increase growth rate and can form part of nutrition strategies to increase overall gut health and pig performance.

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CHAPTER 1

INTRODUCTION

The livestock industry has come under increasing scrutiny for using antibiotics sub-therapeutically as growth promoters. Increased awareness of the negative effects of microbial antibiotic resistance has amplified consumer pressure to raise livestock without the use of these growth promoters (Liu *et al.*, 2018). With the first ban of antibiotic growth promoters (AGPs) in Europe and recently in the USA (2017), the livestock industry is now forced to find viable alternatives to relieve growing consumer concern, improve feed efficiency, lowering pathogenic intestinal and environmental bacterial loads and to delay further development of antibiotic resistant bacteria. This has to be achieved in a more effective and holistic manner to be able to provide safe food for an estimated population of 9 billion people by 2050, without compromising animal performance, health status and profitability of the livestock industry (FAO, 2016). Enhancing feed efficiency is of crucial importance to achieve this difficult task. It is also vital for the success and productivity of the pig production unit. Furthermore, it entitles effectively converting feed mass into pork carcass mass (Patience, 2012; FAO, 2016).

In order to produce pork in an AGP free environment, the producer will often be forced to make changes in management and nutritional strategies in order to avoid increased mortalities and financial losses (Pettigrew, 2006). The aim of these drastic changes is to improve the animal's ability to prevent disease from pathogenic bacteria to enter and proliferate in the host gastrointestinal tract (GIT) from external sources. Furthermore, in order to improve the metabolic utilisation of dietary nutrients offered to the pig, a healthy gut is crucial for the development of the gut epithelial layers and this results in improved feed efficiency and nutrient absorption. The GIT of all animals is inhabited by a complex population of hundreds of species of microorganisms often referred to as the microbiota of the host. The balance and health of this symbiotic population is of crucial importance to the host (Leser *et al.*, 2002).

There is a range of strategies that can aid the nutritionist to achieve pork production in an AGP free environment. Feed additives and more specifically probiotics could act as an alternative to sub therapeutic AGP use. Other strategies include a more holistic approach like the strategic use of dietary fibre in diet formulations due to the potential benefits on gut health, animal welfare and the use of cereal by-products that can also lead to a reduction in diet costs (Jha & Berrocoso, 2015). Strategies outside the field of nutrition that promote the elimination of resistant bacteria include phage therapy, lysin therapy and bacteriocins (Rios *et al.*, 2016). Overcoming antimicrobial resistance may require the application of more than one nutritional strategy. The producer should look at combining different nutritional and health strategies to provide effective solutions against resistant pathogenic bacteria.

Probiotics or DFMs can be described as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" (FAO, 2016). The health benefits that exist with the use of probiotics include firstly, improving and maintaining a healthy gut microflora through competitive exclusion of pathogenic bacteria (Rios *et al*., 2016); secondly, the production of antimicrobial substances like short chain fatty acids (Oelschlaeger, 2010) and thirdly, immunomodulation of the GIT where probiotics affect the innate and adaptive immunity (Hong *et al*., 2005). In order to improve the metabolic utilisation of dietary nutrients offered to the pig, a heathy GIT is very important to ensure feed efficiency. This will lead to improved nutrient absorption for growth through the nutrient uptake of the gut epithelial layers (Liao & Nyachoti, 2017).

Apart from these potential health benefits, DFMs also offer nutritional advantages. Commercial pig diets consist mostly of plant carbohydrate sources. These raw materials are the most abundant nutrient source worldwide and account for more than 70% of feed dry matter as well as 60-70% of the energy intake of pigs (Knudsen, 1997; Bach Knudsen *et al*., 2012). The energy component of growing pigs makes up the most expensive part of the cost contribution to diets, regardless if the energy comes from cereal grains, fat or oil. A wide variety of complex molecular structures make up the non-starch polysaccharide (NSP) fraction of plant carbohydrate sources, containing structurally bound nutrients within the fibre matrix that is unavailable to the monogastric animal (Kerr & Shurson, 2013). Between 10-25% of the NSP or fibre fraction is left undigested as pigs do not synthesise the digestive enzymes necessary to utilise the nutrient containing molecules (Bedford & Partridge, 2010).

Undigested feed components move down the GIT and undergo proteolytic and/ or cellulolytic bacterial fermentation depending on the feed composition and formulation. Diets containing high amounts of NSP tend to increase digesta viscosity. This in turn decreases endogenous enzyme digestion efficiency and increases the nutrient competition between the host and beneficial/ pathogenic gut microbiota (Latorre *et al.*, 2016). Spore-forming bacteria, in particular from the genus *Bacillus*, seem to be a promising alternative to reduce the NSP content of feeds due to the ability to produce a range of different exogenous enzymes which differs between different *Bacillus* species (Bedford & Partridge, 2010., Larsen *et al.*, 2014; Zaghari *et al.*, 2015., Latorre *et al.*, 2016). It is therefore important to comprehend that there are significant differences between different *Bacillus* species and further more complex differences between strains of the same species. In-feed supplementation of *Bacillus* species has resulted in numerous performance improvements in pigs. Improved weight gain, feed efficiency and mortality were noted in weaned piglets (De Lange *et al.*, 2010; Le Bon *et al.*, 2010; Patil *et al.*, 2015; Jørgensen *et al.*, 2016), while improved digestibility, growth performance, feed conversion and carcass quality were shown for growing pigs (Alexopoulos *et al.*, 2004; FAO, 2016; Jørgensen *et al.*, 2016; Londoño *et al.*, 2016). Although a number of studies have shown that probiotics have promising effects on animal production, results tend to be highly variable and inconsistent (Kornegay & Risley, 1996; Meng *et al.*, 2010; Wang & Gu, 2010). The combination of different selected strains of probiotic organisms could have a

synergistic effect in their properties and multi-strain products have become increasingly popular (Zimmermann *et al.*, 2016).

The aim of this study was to evaluate the effects of a spore-forming probiotic (Bioplus YC, Chr. Hansen Denmark) on the nutrient availability of pig feeds and whether the specific probiotic is able to release additional energy (0.3 MJ/kg or 72 kcal/kg on net energy level) from the undigested or unabsorbed dietary fibre fraction. To achieve the aim, the performance of pigs receiving standard and reduced energy diets, with and without a commercially available probiotic feed additive, was measured.

The null hypothesis (H_0) of this study was that probiotic supplementation does not improve feed efficiency and production performance parameters of commercial pigs and pig diets. The alternative hypothesis (H_1) was that probiotic supplementation improves feed efficiency and production performance parameters of commercial pigs and pig diets.

A second null hypothesis (H_0) of this study was that probiotic supplementation is unable to contribute 0.3 MJ/kg or 72 kcal/kg on net energy level from the dietary fibre fraction. The alternative hypothesis (H_1) was that probiotic supplementation is able to at least contribute 0.3 MJ/kg or 72 kcal/kg on net energy level from the dietary fibre fraction.

CHAPTER 2

LITERATURE REVIEW

2.1 Carbohydrates and fibre in the pig diet

Commercial pig diets are mostly made up of plant carbohydrate sources which account for approximately 60-70 % of dry matter in the diet. These plant sources also contribute to roughly two-thirds of the total dietary energy intake of the animal (Jha & Berrocoso, 2015). Most of the ingredients in the pig diet comprise of cereal grains, which also supply most of the energy and is made up of different concentrations of carbohydrates, fibre, lipids and crude protein that differs in its composition (Stein *et al.*, 2016). The structures in which these nutrients are stored are complex and an understanding of the molecules these nutrients are bound to, is important for effective diet formulation.

Dietary carbohydrates are present in many forms over various feed raw materials and are primarily classified by its molecular size or degree of polymerisation. The type of linkage on the carbohydrate chain, either being α- or β- form and the composition of the sugar monomers (i.e. trioses, pentoses and hexoses) further contributes towards its specific classification. These classification criteria divide carbohydrates into 3 groups namely sugars, oligosaccharides and polysaccharides (Bach Knudsen *et al.*, 2012, 2016). Sugars consist of monosaccharides (monomers of carbohydrates), disaccharides and sugar alcohols. Oligosaccharides are a combination of two, three or four monosaccharides that form a larger molecule and can be further divided into either α-glucans (i.e. malto-oligosaccarides), which is present in enzymatically hydrolysed starch, or non α-glucan molecules that include α-galactosides like faffinose, stachyose, fructo- and galacto-oligosaccharides. Polysaccharides are by far the largest component of carbohydrates found in swine diets and can be divided into starch that is in the form of α-1,4 as well as α-1,6 glucans and lastly non-starch polysaccharides or NSP (Bach Knudsen *et al.*, 2016). These molecules contain a high molecular weight and are composed of large numbers of pentose or hexose residues. Many of these complex structures occur in plants either as reserve food material such as starch, or serve as structural components like cellulose (McDonald, 2011).

Plants mostly store carbohydrates in the form of starch and generally consist of glucose molecules containing 15-30% of amylose and amylopectin. The part that contains amylose consist of a non-branched helical chain of glucose molecules linked by α-1,4 glycosidic bonds. Amylopectin on the other hand has a high molecular weight and highly branched polymers linked together with α-1,4 as well as α-1,6 glycosidic bonds (Bach Knudsen *et al.*, 2012, 2016). The ratio of amylose to amylopectin differs between different feed ingredients and sources of the same feed ingredients. In most starches, amylopectin is the main component, which constitutes 70-80 % of total starch content, with amylose typically comprising 15-30% of most common starches (McDonald, 2011, Bach Knudsen *et al.*, 2016).

The starch particles in cereal grains are classified under glucans and are embedded in a complex protein matrix surrounded by cell wall components. This matrix is hydrophobic and obstructs enzyme digestion by α-amylase (Bach Knudsen *et al.*, 2016). Normally, the starch in cereal grains is more digestible than the starch found in legumes. Pigs have the ability to digest starch very efficiently even though there are differences in starch and amino acid (AA) digestibility of different dietary ingredients (Stein *et al.*, 2016).

The term 'dietary fibre' is often referred to by nutritionists as the polysaccharides of plants that cannot be hydrolysed by the digestive enzymes of more complex animals even though these structures contain nutrients that can benefit the host. These complex plant polysaccharides include cellulose, hemicellulose, pectin substances, fructans, β-glucans and lignin (Fuller, 2004). An illustration of plant carbohydrate classification can be seen in Figure 1 (Hall, 2003). Cellulose is known to be the most abundant single polymer in plants and plays a crucial role in the structure of plant cell walls. Cellulose is a homoglycan that is high in molecular weight and β-glucose molecules are 1,4-linked. Cellulose structures are formed by ordered aggregate compaction of cellulose molecules, which are held together by inter- and intramolecular hydrogen bonding. Cellulose in plant cell walls is chemically and physically closely associated with hemicellulose and lignin (McDonald, 2011). Hemicellulose is defined as the alkali-soluble cell wall polysaccharides that are closely associated with cellulose (McDonald, 2011). These molecules are mostly made up of mixtures of xylans, glucomannoglycans, arabinogalactans, arabinans and arabinoxylans (Fuller, 2004). Hemicellulose from plants contains a main chain of xylan made up of β-(1:4)-linked D-xylose units, which contain glucose, galactose and arabinose on the side chains. The pig has the ability to digest as much as 50% of the cellulose and hemicellulose fraction of cereal grains and their by-products due to microbial and hindgut fermentation (McDonald, 2011). Ligin is an insoluble polyphenolic compound which is not a polysaccharide, but is closely associated with this carbohydrate group of compounds. Ligin is indigestible by all animals and it is negatively correlated with the digestibility of the cell wall component (Fuller, 2004). Pectic substances are polysaccharides that form part of the heteroglycan group that is soluble in hot water and occur as constituents of primary cell walls and intercellular regions of higher plants. The primary structural component of pectic substances is a chain of 1,6-linked residues of α-D-galacturonic acid and from this primary chain, extensive modification can occur (Drochner *et al.*, 2004). Fructans can be defined as polysaccharides of β-D-fructofuranose residues, with a non-reducing terminal D-glucopyranose, synthesised from sucrose and common fructose molecules and include inulin as well as levan (Fuller, 2004). All fructans are soluble in cold water and contain a relatively low molecular weight. Hydrolysis of fructan molecules yields D-fructose and a small amount of D-glucose, which is derived from the terminal sucrose unit in the fructan molecule (McDonald, 2011).

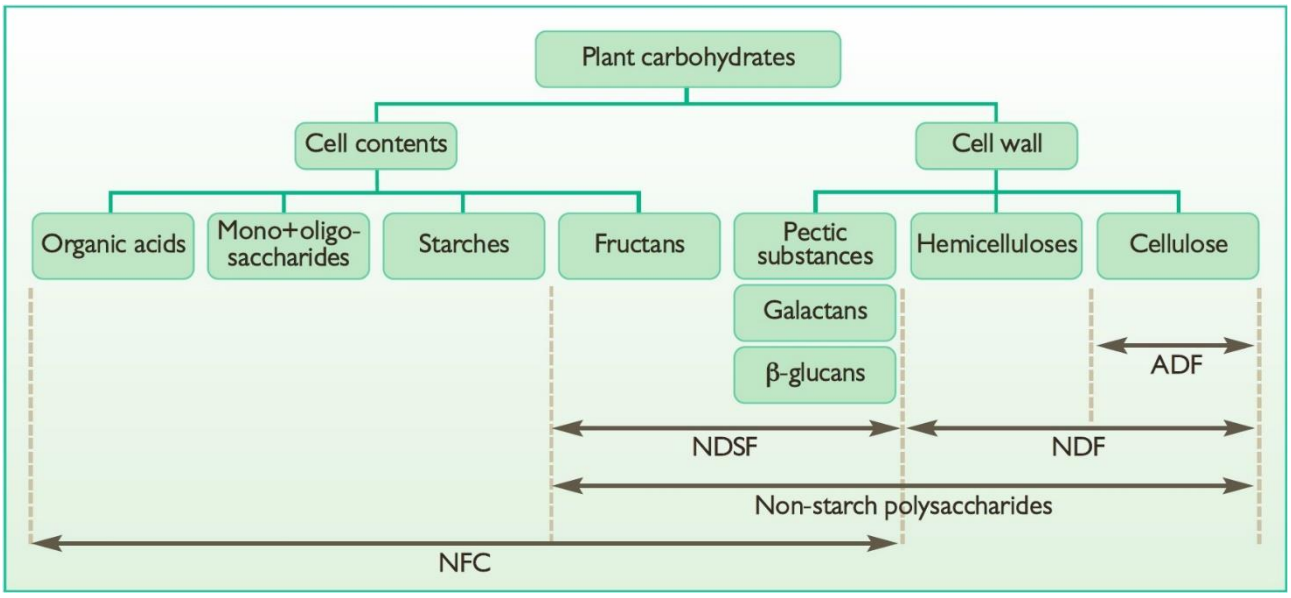

Figure 2.1. Illustration of the classification of plant carbohydrates. NSP = Non starch polysaccharides. NDF = neutral detergent fibre; NDSF = neutral detergent-soluble fibre; NFC = non-NDF carbohydrates; ADF = Acid detergent fibre (Hall, 2003)

Complex dietary fibre structures provide a major source of energy (60-80%) to the ruminant animal in the form of short chain fatty acids (SCFA). These SCFAs are a direct result of ruminal fermentation by anaerobic rumen microbiota that yield acetic, propionic, butyric and lactic acid as well as carbon dioxide, methane and hydrogen (McDonald, 2011). The rumen microbiota is made up of an estimated population of 400-600 different strains of bacteria living in symbiosis with the ruminant animal and is able to produce a range of enzymes that are able to catabolise and degrade these complex fibre structures or polysaccharides (Fuller, 2004). Starch present in the diet will also undergo ruminal fermentation in the ruminant, whereas starch is enzymatically hydrolysed and digested in the stomach and small intestine of the non-ruminant. More complex dietary fibre is mostly fermented in the hindgut (caecum and large intestine) and to a lesser extent fermented by microbiota in the small intestine (Fuller, 2004).

As a result of physiological differences between ruminants and monogastric animals, significant changes in formulation strategy is required in order to provide diets that allow animals to grow to their genetic potential. With the absence of ruminal fermentation in monogastric animals, an understanding of complex polysaccharides and their effect on the gastrointestinal tract (GIT) of the pig is vital to enhance feed efficiency. Plant polysaccharides contain large amounts of nutrients that could enhance growth performance and feed efficiency (Bach Knudsen *et al.*, 2016).

2.2 Fibre digestion and energy utilisation in pigs

The efficiency of degradation and the utilisation of starch in plant fibre is generally better fermented in the ruminant than in the caecum and large intestine of the pig or any other non-ruminant animal (Fuller, 2004). In normal circumstances, the digestibility of dietary fibre is lower (40-60%) than nutrients like starch, sugar, fat, and crude protein (around 80%) and the digestibility of nutrients are negatively affected by increasing the percentage of dietary fibre (DF) in the ration. This is to such an extent that digestible energy content is negative and linearly affected by the increase of dietary fibre in pigs (Jha & Berrocoso, 2015). The energy content of the diet is known to be the main determinant of voluntary feed intake in pigs of all the nutrients used in the feed (Lan *et al.*, 2017). The age and physiological stage of the pig also play a crucial role. DF is better and more efficiently digested in adult sows (by an average of 0.6 MJ/kg dry matter) than growing pigs (Noblet & Perez, 2014; Jha & Berrocoso, 2015). This is mostly due to the higher rate of fibre degradation in the hindgut of adult sows as well as a longer retention time of digesta and lower feed intake per kg live weight.

Plant fibre makes up a large part of the pig diet and a certain part of this plant carbohydrate source is left undigested by the secreted digestive enzymes of the monogastric animal. This fraction becomes available to the microbiota colonising various parts of the gut, to undergo bacterial fermentation. It is also this fraction of dietary fibre that reduces dietary nutrient and energy digestibility due to its physio-chemical properties that include solubility, viscosity and water holding capacity in monogastric animals (Jha & Berrocoso, 2015). The efficacy of DF polysaccharide fermentation depends on the physical and biochemical characteristics of the plant material. Extensive lignification of the dietary ingredients will restrict fermentation and reduce digestion as well as absorption efficacy (Brownlee, 2011; Bach Knudsen *et al.*, 2016). The solubility of the DF source also play an important role as unlignified and soluble pectans, hemicelluloses, β-glucans and fructans will be fermented in the large intestine of the pig (Fuller, 2004). Dietary ingredients containing high amounts of insoluble fibre are mostly resistant to microbial degradation. Consequently, the non-degraded fractions increase faecal dry matter content or bulkiness and reduce feed efficiency (Jha & Berrocoso, 2015).

The DF fraction and its various forms not only influence the digestibility of other nutrients, but also affect the physiological functions of the gut. These effects are mainly determined by the level and type of fibre together with the water holding capacity, solubility and viscosity of the fibre source (Bach Knudsen *et al.*, 2016). The soluble fraction of DF is known to increase digesta viscosity, which tends to limit penetration of endogenous digestive enzymes in digesta contents, creating an unstirred water layer on the intestinal surface. This creates a physical barrier that limits digestion efficiency, causing a reduction in nutrient digestion and absorption. The second physiological effect of DF on the GIT is an increase in endogenous nitrogen losses (Knudsen, 1997; Bach Knudsen *et al.*, 2012, 2016). This again depends on the DF type, especially the water holding capacity of the soluble dietary fibre. As the digesta viscosity increases, the gut chyme stimulates epithelial cell proliferation and this could contribute to the loss of endogenous cells (Jha & Berrocoso, 2015).

In weaned pigs, the effect of increased digesta viscosity causes villus atrophy as a result of more aggressive cell exfoliation and deeper crypt depths. This is also known as the mechanical action of dietary fibre and compromises the mucin layer, which poses as a risk for damage from opportunistic pathogenic bacteria (Montagne *et al.,* 2003). The strategic use of fibre in different life stages of the pig is thus vital towards sufficient organ development and growth.

Although most of the DF escapes enzymatic breakdown in the pig, it is possible that a substantial amount of DF can be utilised by fibre degrading enzymes of microbial origin in the stomach and small intestine. This leads to a partial disruption of cell wall components. Compared to glucose, a reduced digestibility of xylose, arabinose and uronic acid were found in the small intestine of piglets on cereal grain and soya-based diets, whereas higher levels of insoluble fibre content on the other hand negatively affects the accessibility and efficacy of enzymatic breakdown of feed components in the small intestine as well as an impaired microbial fermentation in the large intestine (Giuberti *et al.*, 2015). High levels of insoluble fibre also increase the faecal DM and bulkiness as a result of the resistance to enzymatic and microbial hydrolysis (Wilfart *et al.*, 2007).

Undigested NSP molecules will reach the large intestine and undergo microbial hindgut fermentation. The order of hindgut fermentation in the large intestine follows a structural fashion where sugar residues and nondigestible oligosccharides are fermented first, followed by starch residues, soluble NSP and insoluble NSP (Bach Knudsen *et al.*, 2016). This process produces SCFA like acetate, propionate and butyrate that are rapidly absorbed in the GIT and contribute 5-28% of the maintenance energy requirement of pigs (Kerr & Shurson, 2013). The extent of hindgut fermentation depends on the conformation of dietary polysaccharides and the structural association with indigestible fractions like lignin; normally the digestion of complex polysaccharides like cellulose are small compared to ruminants that have adapted to utilise these sources more efficiently (McDonald, 2011). During normal hindgut fermentation patterns, acetate is considered to account for approximately 66% of the produced SCFA followed by propionate and butyrate. Many bacterial groups are responsible for producing acetate, but only a few groups produce propionate and butyrate as end products of fermentation (Louis *et al.*, 2007). Between 95-99% of produced SCFA is absorbed before reaching the rectum and the individual SCFA are used in different ways within metabolism cycles. Acetate and propionate are both transported to the liver and respectively utilised as an energy substrate for muscle tissue while the other is converted to glucose. Butyrate is primarily used by epithelial cells in the colon as a major source of energy for metabolic activities (Montagne *et al.*, 2003). Apart from being a source of energy, butyrate is regarded as an important metabolite due to several cellular related effects like cell development stimulation, growth and renewal of intestinal cells, reduction of epithelial cell apoptosis and a possible improvement in colon mucosal integrity (Giuberti *et al.*, 2015).

2.3 The non-starch polysaccharide fraction in pig diets

The use of more affordable dietary raw materials, like cereal grain by-products, has led to an overall reduction in digestibility of many dietary nutrients in pig diets. These alternative sources, for example wheat, sorghum, barley and their co-products have led to a dietary cost saving, but increased the amounts of anti-nutritional factors (ANF), including the NSP fraction, that could negatively impact growth and performance of animals when compared to maize-based diets (Latorre *et al.*, 2016; Wu *et al.*, 2018).

The NSP fraction of diets can be defined as the polymeric fraction of dietary fibre that includes all polysaccharides except lignin and starch (Fuller, 2004). NSP molecules are classified in one of three chemically definable groups: cellulose, non-cellulosic polysaccharides and pectic polymers. Non-cellulosic polysaccharides include, but are not limited to, arabinoxylans (pentosans), mixed-linked β-glucans, mannans, galactans, xyloglucans and fructans (Choct, 2015). These molecules are not hydrolysed by the endogenous enzymes secreted by the non-ruminant host and this fraction becomes available as a substrate for microbial fermentation in the large intestine (Jha & Berrocoso, 2015). Based on potential nutritional value, the NSP fraction of pig diets can be divided into two main categories, with different nutritional properties and effects on the host. The two main NSP fractions are soluble NSP and insoluble NSP, which will be discussed below.

The solubility of an NSP molecule normally refers to water solubility and can be regarded as an important measure of the physico-chemical characteristics and nutritional properties of a soluble NSP molecule for use in pig nutrition. There is a negative relationship between the amount of soluble NSP and the nutritive value of the diets in pigs. The negative effects of large amounts of soluble NSP can be overcome by the use of appropriate feed additives like xylanase enzymes (Choct, 2015). Insoluble NSP includes the fraction that is insoluble in water. The solubility of the NSP play an important role in determining if a polymer could act as an ANF, for instance, arabinoxylans found in rye and wheat as well as the β-glucans in barley and oats are mostly soluble. The arabinoxylans found in maize and sorghum are of lower solubility than other ingredients (Jha & Berrocoso, 2015). The majority of fibre in wheat and maize consist of arabinoxylans and cellulose but the total tract fermentability is better and more efficient in wheat than maize (Stein *et al.*, 2016). The insoluble fraction of total DF in maize and maize by-products is largely resistant to fermentation in the hindgut and only 40% of this part of the DF is fermented in the GIT of pigs (Jha & Berrocoso, 2015). Table 2.1 provides some information on the types and levels of fibre components in some common cereal grains and their co-products.

Table 2.1. Types and levels of fibre components in some common cereal grains and co-products (g/kg DM) (Jha & Berrocoso, 2015)

		Barley		Oats								
	Wheat	Hulled	Hulless	Hulled	Hulless	Maize	Sorghum	Rye	Wheat bran	Wheat middlings	DDGS	Sugar beet pulp
Starch	618	587	645	468	557	620	690	613	222	168	86	5
Cellulose	13	39	10	82	14	17	15	15	72	67	58	203
NCP												
Soluble	19	56	50	40	54	25	4	42	29	12	34	290
Insoluble	62	88	64	110	49	38	47	94	273	227	158	207
NSP												
Arabinoxylans	81	12	48	98	36	17	17	89	238	52	61	165
β-glucan	8	43	42	28	41	6	1	20	24	21	63	8
Mannose	2	2	4	3	3	2	1	3	5	19	9	8
Galactose	3	2	3	7	4	8	3	3	9	13	14	38
Uronic acids	4	2	2	10	5	8	4	2	15	16	16	199
Total NSP	95	167	124	232	116	81	66	132	374	250	192	700
Lignin	18	35	9	66	32	8	16	21	75	39	32	37
Dietary fibre	112	202	133	298	148	89	83	153	449	289	322	737

DM = dry matter; NCP = non-cellulosic polysaccharides; NSP = non-starch polysaccharides; DDGS = distillers dried grains with solubles

As the soluble part of the NSP content solubilise, the consequence of increased luminal viscosity is recently known to affect the intestinal mucus by the binding of NSP to the protective and lubricating film. This visco-elastic gel is important to protect the host against normal and pathogenic bacteria and furthermore it is also involved in the nutrient absorption as well as transport across the gut epithelial layer into the blood (Bach Knudsen *et al.*, 2012, 2016). A further consequence of the interaction of soluble NSP and the mucus layer of the gut epithelium layer is the higher amounts of endogenous losses found with feeding increased amounts of soluble DF (Bach Knudsen *et al.*, 2012). The soluble NSP fraction adds increased pressure on the pancreas and the mucosal surface for additional secretions and bile production due to the ANF of the specific NSP fractions present in the digesta. This could also influence feed intake through negative feedback mechanisms involving cholecystokinin and gastric inhibitory polypeptide production, which are both involved in negative feedback systems on pig satiety (Cadogan & Choct, 2015). Furthermore, with increased levels of NSP content in the stomach, higher viscosity levels in the digesta reduce the sieving ability of the stomach. This involves larger particles that are kept in suspension and unable to descend to the bottom of the stomach, prohibiting proper digestion of these particles as well as the flow of digested nutrients to the duodenum where the fraction of undigested nutrients could increase (Ellis *et al.*, 1996). The viscosity associated with soluble NSP is not related to the linkage type or sugar composition of the molecule, but rather physical properties of the polysaccharide, such as molecular weight, distribution and structure (Choct, 2015).

Cadogan & Choct (2015) indicated that it could be possible for older pigs to better alleviate the negative effects of NSP due to the better developed and longer GIT, as well as a more stable gut microbiota. In young pigs weighing more than 20 kg, compounds like lectins, antigens and oligosaccharides no longer have such a negative effect as in the newly weaned piglet, as the fermentation of these particles is adequate. In the young piglet weighing less than 20 kg, these compounds are problematic and cause reduced efficacy of digestion (Stein *et al.*, 2016). Therefore, the age of the pig is an important factor to consider, as the digestive ability of the young pig to utilise dietary fibre is not fully developed and undigested fractions could have negative effects on growth performance.

The pig is not able to secrete endogenous enzymes that are able to hydrolyse β- bonds in oligosaccharides or even monosaccharides that are found in NSP. Throughout the small intestine, non-digestible oligosaccharides are hydrolysed 40-95% and NSP are only degraded 20-25%. It is most probably achieved by microbial enzymes, produced by microbiota present in the small intestine (Bach Knudsen *et al.*, 2016). Degradation by microbial enzymes in the small intestine alter the structure and size of the NSP molecule. This enables microbiota in the large intestine to ferment and further degrade the partially degraded NSP particles more efficiently (Knudsen, 1997; Bach Knudsen *et al.*, 2016). As DF exits the small intestine it will supply the majority of carbohydrates in the large intestine together with mucopolysaccarides and exfoliated epithelial cells (Bach Knudsen *et al.*, 2016). Microbial degradation of non α- carbohydrates in the large intestine occurs in the following order: sugar residue and non-digestible oligosaccharides, starch residues, soluble NSP and lastly resistant starch or insoluble NSP (Giuberti *et al.*, 2015; Bach Knudsen *et al.*, 2016). Almost all sugars, non-digestible oligosaccharides, residual starch and soluble NSP are degraded by active microbial degradation in the caecum and upper part of the colon.

Non-digestible oligosaccharides (fructo-oligosaccharides, transgalacto-oligosaccharides), inulin and lactulose are utilised by the microbiota in the large intestine and act as prebiotics. Prebiotics are defined as 'a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth or activity of one or a limited number of bacteria in the digestive tract that has the potential to improve host health' (Fuller, 2004). This is done by the stimulation of cellulolytic bacteria that increases the concentration of lactic and acetic acid in the large intestine. As a consequence, the pH of the large intestine decreases, further favouring cellulolytic bacteria (Liu *et al.*, 2018). The environment created by prebiotic substances is unfavourable for pathogenic proteolytic bacteria due to the lower gastric pH conditions. The beneficial effects arising from prebiotics in pig diets include increased fermentability and subsequent synthesis of SCFAs resulting in the reduction of intestinal pH and ultimately lowering protein fermentation. When carbohydrates are in short supply relative to protein in the large intestine, protein fermentation will commence, thus favouring a different range of microbiota and fermentation end-products which would include NH_3, branched-chain VFA as well as potentially toxic end-products that include amines, volatile phenols and indoles (Lallès *et al.*, 2007a).

Therefore, preference towards cellulolytic fermentation balance in the large intestine could be a useful strategy to improve GIT health. Insoluble NSP like arabinoxylans, xylans and cellulose are degraded much slower due to cross linkages of cell wall polysaccharides to lignin (Bach Knudsen *et al.*, 2016). The native microbiota population is therefore an undeniable factor in the hydrolysis and utilisation of complex plant polysaccharides that could ultimately benefit the host.

2.4 Enzyme secretion in growing pigs

The introduction of enzymes to the digestion process starts as early as in the mouth of the animal, where large particles of food are broken up into smaller pieces and mixed with saliva. Saliva is made up of approximately 99 per cent of water, with the remaining one per cent consisting of mucin, inorganic salts, the enzymes α-amylase and the complex lysozyme (McDonald, 2011). Although the activity of α-amylase is low in the mouth and oesophagus and some digestion does occur, the pH of the stomach is unfavourable for α-amylase activity. The pH optimum for α-amylase is slightly lower than the pH of the saliva, which is normally around 7.3. The α-amylase enzyme hydrolyses the α-(1,4)-glucan links in polysaccharides containing three or more α-(1,4)-linked D-glucose units. The enzyme hydrolyses starch, glycogen, polysaccharides and oligosaccharides. Breakdown of amylose, which contains mostly α-(1,4)-glucosidic bonds, produces a mixture of glucose and maltose molecules. Another starch molecule, amylopectin is linked by α-(1,4)-glucosidic bonds and a number of branched α-(1,6)-glucosidic bonds, which are not hydrolysed by α-amylase. The products of amylopectin hydrolysis is a mixture of branched and unbranched oligosaccharides which contain a large amount of α-(1,6) bonds (Bach Knudsen *et al.*, 2016).

As digesta enters the stomach, gastric juices are secreted to digest complex protein sources in an acidic environment, also to eliminate potential pathogenic bacteria ingested with the diet (Efird *et al.*, 1982). The gastric juice consists of water, pepsinogens, inorganic salts, mucus, hydrochloric acid and the intrinsic factor important for the efficient absorption of vitamin B_{12}. Pepsinogens present in the gastric juice are inactive upon secretion and activated by hydrochloric acid, converting the pepsinogens to pepsin, which hydrolyse those peptide bonds adjacent to aromatic amino acids, e.g. phenylalanine, tryptophan and tyrosine, but they also have a significant action on linkages involving glutamic acid and cysteine. Pepsin is also important in the clotting action of milk, together with rennin that is present in the gastric juice of piglets. The products of high acidic enzyme hydrolysis in the stomach are mainly polypeptides of variable chain length and a few amino acids (McDonald, 2011).

As partially digested stomach contents move to the duodenum section of the small intestine, it is mixed with duodenal, pancreas as well as liver secretions. Brunner's glands, located in the upper part of the duodenum, secrete an alkaline substance that lubricates and protects the duodenal wall from the low pH

contents coming from the stomach. Bile is produced by the liver and enters the duodenum via the bile duct; it consists of sodium and potassium salts of bile acids, mainly glycocholic and taurocholic, phospholipids, the bile pigments biliverdin and bilirubin, which are the end products of haem catabolism, cholesterol and mucin. These compounds play a crucial role in the activation of pancreatic lipase and the emulsification of dietary fats to be absorbed further down the small intestine (Lindemann *et al.*, 1986; Jensen *et al.*, 1997).

The pancreas is a gland located in the duodenal loop that secretes a watery fluid containing a high concentration of bicarbonate ions in the proximal part of the duodenal loop, and further down the secretion of pro-enzymes and enzymes such as trypsinogen, chymotrypsinogen, procarboxypeptidases A and B, proelastase, α-amylase, lipase, lecithinases and nucleases occurs. These enzymes have a pH optimum of 7-9. The hydrolysis action of pancreatic α-amylase is similar to that of salivary α-amylase, thus breaking down α-(1,4)-glucan links found in glycogen and starch. Hydrolysis of dietary fats is achieved by pancreatic lipase. The breakdown action of triacylglycerol by pancreatic lipase is not complete and stops at the monoacylglycerol stage, further breakdown and absorption rely on the process of emulsification. Monoacylglycerol and most fatty acids dissolve and emulsify in the secreted bile salts to form pure bile salt micelles and mixed micelles (Lindemann *et al.*, 1986; Jensen *et al.*, 1997; Rantzer *et al.*, 1997). Lecithinase A hydrolyses the bond between a fatty acid and the β-hydroxyl group of lecithin to form lysolecithin; this is further hydrolysed by lysolecithinase to form glycerolphosphocholine and a fatty acid. Nucleic acids (DNA and RNA) are catalysed at the cleavage of the ester bonds between phosphoric acid and sugar molecules by polynucleotidases deoxyribonuclease (DNase) and ribonuclease (RNase) to yield nucleotides. The further breakdown of oligosaccharides to monosaccharides, as well as peptides to amino acids is by the action of enzymes secreted by the intestinal epithelial cells (McDonald, 2011).

Enzymes secreted by the intestinal villi that hydrolyse monosaccharides include sucrase, which converts sucrose to glucose and fructose; lactase, which catabolises lactose to glucose and galactose and 1,6-glucosidase, that hydrolysis α-(1,6) links in dextrins. Lastly aminopeptidases attack the peptide bonds situated next to the free amino group of simple peptides and dipeptides are broken down to amino acids by dipeptidases. A certain part of the diet is fermented by microbial organisms before the large intestine (McDonald, 2011).

The development of the GIT during ontogenesis in the prenatal piglet is a complex and ongoing process that starts prenatally and continues long after birth, with the diet playing an integral part of the structure and functional development of the GIT. A major problem experienced by modern pig producers is the early weaning of piglets and subsequent poor performance during the transition period, hence changing from a liquid diet (provided every 60-90 minutes) to dry feed (fed *ad libitum*) (Efird *et al.*, 1982). In order to formulate effective diets for the successful transition from one phase to the next during the early weaning period, it is crucial to know and understand the physiological aspect and capabilities of the digestive system during and

after this most sensitive period. As a result of the weaning process, change in the GIT is inevitable (Efird *et al.*, 1982). The first ingestion of colostrum causes an increased acceleration in small intestine growth of nearly 30% in the first three days of age and weight of this organ nearly doubles at the same time. Crypt depth and villi height also increase by 40% and 35% respectively, in the first three days of age (Barszcz & Skomiał, 2011). Research by Efird *et al.* (1982) found that one-day old piglets already had the ability to acidify stomach contents and secrete hydrochloric acid to a pH of 2 when raised in a sanitary environment. Results of this study suggest that the pH of stomach contents were higher in artificial diets when compared to piglets reared off the sow. Furthermore, artificial weaning diets increased the stomach pH and increased pepsin secretion, which has a working pH optimum of 2 and 3.5. Activities of trypsin and chymotrypsin, at weaning on dry diets, steadily increased between 16 and 22 days of age. When compared to piglets weaned off the sow, total trypsin and chymotrypsin activities tended to be lower in piglets receiving a dry diet.

During weaning the GIT has to adapt to the new plant-based diet, which induces changes in the myenteric motility, enzyme secretion and enzyme activity, as well as the composition of the present microbiota. The onset of weaning induces a reduced activity of brush border enzymes (Barszcz & Skomiał, 2011). A characteristic feature of weaning stress is also a rapid decline of pancreatic enzyme activity, which contributes to reduced nutrient digestibility and absorption in the first week after weaning (Lindemann *et al.*, 1986; Barszcz & Skomiał, 2011). During the weaning period the qualitative composition of the gastric juice also change. For example, foetal type hydrolyses is replaced with new isoforms of enzymes, like protease E and elastase 1. A time period of approximately two weeks after weaning is required to restore enzyme activity to the level it was before weaning and the time required to stabilise the pancreatic enzyme activity depends on the protein source used in the diet, as well as the level of feeding interval (Barszcz & Skomiał, 2011). In a study by Kelly *et al.* (1991), the change in pancreatic enzyme secretion on 3, 5 and 7 days in force-fed, post-weaning piglets were measured and compared with piglets that were left suckling on the sow till 14 and 21 days of age. Results indicated a 50% reduction in lactase activity 7 days post-weaning. All weaned groups indicated an increase in specific sucrase activity over non-weaned 14-day old sow-reared piglets. Even though the sucrase activity increased in weaned vs. non-weaned piglets, there was also a drop in sucrase activity 7 days post-weaning, but not as severe as the 50% drop in lactase activity. Dramatic increases in maltase and glucoamylase were measured 3 days post-weaning as a result of rapid substrate induction, as the weaner diet contained increased amounts of cereal carbohydrates and much lower levels of lactose than found in sow milk (Kelly *et al.*, 1991).

The main site of hydrolysed dietary nutrient absorption is the small intestine and most of these nutrients are absorbed by the terminal ileum. Nutrients undigested by enzymatic action of the small intestine, migrate to the large intestine where retrieval of nutrients, electrolytes and water occurs. Polysaccharides such as cellulose, hemicellulose and lignin are unaffected by digestive enzymes secreted by the monogastric animal and enclose valuable protein and carbohydrates that is not absorbed by the host (Louis *et al.*, 2007). The large

intestine itself does not have glands that produce enzymes and substances secreted originates mostly from mucous glands that line the large intestine. Enzymatic activity in the large intestine is brought about by enzymes carried down from the small intestine or as a result of microbial fermentation and microbial enzyme secretion. The large intestine hosts a diverse population of microbiota and an extensive microbial activity including organisms like lactobacilli, streptococci, coliforms, bacteroides, clostridia and yeasts (Louis *et al.*, 2007; Brownlee, 2011). These organisms are able to metabolise a wide array of carbohydrate and nitrogen from the dietary residues resulting in the production of indole, skatole, phenol, hydrogen sulphide, amines, ammonia and volatile fatty acids (acetic, propionic and butyric). Cellulose and other complex polysaccharides not hydrolysed by the pig's enzymes undergo digestion by microbial fermentation, but is not as complete or effective as ruminants whose digestive systems are adapted to flourish on fibrous diets (McDonald, 2011).

It could therefore be concluded that the enzymatic system is fairly limited in monogastric animals in terms of digestion of more complex plant carbohydrate molecules. Increased hydrolysis of dietary fibre could provide additional nutrients and increase feed efficiency. The role of microorganisms in the breakdown of dietary ingredients in the GIT is significant and the correct balance of microbiota populations could aid the enzymatic system in more effective hydrolysis of dietary carbohydrates. An understanding of the microbiota populations and their function in the GIT of the pig is therefore necessary.

2.5 The microbiota population of growing pigs

The GIT of the pig hosts a diverse community of organisms and has a symbiotic relationship with the host. The balance in this ecosystem is of crucial importance in maintaining nutritional, physiological and immunological functions within the host (Fouhse *et al.*, 2016). Microbial fermentation accounts for the disappearance of 8-16 percent of organic matter in conventional pig diets and the effect the microbiota has on the host and its nutrient utilisation is undeniable (McDonald, 2011).

In the newly born piglet, colonisation of intestinal organisms starts immediately after birth. The organisms that colonise the GIT depends on the exposure and repeated environmental exposure that the piglet is subjected to (Fouhse *et al.*, 2016). Organisms that initially colonise the gut include *E.coli* spp. and *Streptococcus* spp. and these create an anaerobic environment for the subsequent colonisers like *Bacteriodes* spp., *Bifidobacterium* spp., *Clostridium* spp. and *Lactobacilli* spp. (Konstantinov *et al.*, 2006). The latter plays an important role in disease prevention and it seems that lactic acid producing bacteria predominates the GIT of the piglet before weaning. This helps to reduce the pH in the gut by the production of lactic acid which helps inhibit enteric pathogens (Li *et al.*, 2001). The bacteria present in the environment therefore has an important role to play in the GIT development of the piglet before weaning.

In modern pork production piglets are weaned at an early age to meet strict production goals, this is done before the piglet establishes a stable microbial population, mature enzymatic system as well as a mature immune system. Enormous stress levels are induced to piglets by removal from the sow together with an abrupt end to nutritious sow milk and placement in a new and unknown environment, all while having an immature and unstable gut ecosystem. These stresses cause an increased risk of diseases due to lowered defences against pathogenic and opportunistic organisms (Fouhse *et al.*, 2016). Early weaning between 21 and 28 days of age is known to trigger an upset in the gut microbiota, which may impair gut physiology and immune function (Konstantinov *et al.*, 2006).

Post-weaning diarrhoea (PWD) is one of the main causes of economic loss in swine production worldwide and is typically identifiable by the occurrence of diarrhoea and a reduction in growth (Fouhse *et al.*, 2016). PWD is caused by a reduction in beneficial lactic acid producing bacteria like *Lactobacillus sobrius, L. acidophilus* and *L. reuteri* and an increase in pathogenic *E. coli* (Konstantinov *et al.*, 2006; Dowarah *et al.*, 2017). The weaning period is associated with low and variable feed and water intake as only 50% of piglets consume their first meal within 24 hours post-weaning and 10% of piglets still not having eaten their first meal at 48 hours post-weaning (Lallès *et al.*, 2007a). While populations of *L. sobrius, L. reuteri,* and *L. acidophilus* remain plentiful and stable before weaning, the population of these organisms drop suddenly after weaning. Early weaning of piglets is then consequently characterised by a compositional and functional instability of the gut microbiota. Furthermore, beneficial Lactobacilli are severely suppressed in the post-weaning period, possibly due to the complex nutritional requirements of these beneficial bacteria, which is affected by the restriction of feed intake around weaning (Lallès *et al.*, 2007b). As PWD sets in, disbiosis of the GIT is also caused by the reduction in feed and water intake leading to anorexia, which changes the structure of the intestine by means of villus atrophy and crypt hyperplasia; this further causes gut barrier dysfunction and increases the risk of disease and mortality (Lallès *et al.*, 2007a). The weaning of piglets, PWD, and the change in the balance of the gut microbiota also change the gut function and intestinal morphology. These changes influence digestion of nutrients as well as the absorption of digested nutrients, which is the consequence of villus atrophy and crypt hyperplasia (Pluske *et al.*, 1997). The producer should thus strive to limit the effects and occurrence of PWD as thoroughly as possible, as it has significant effects on herd performance and profitability.

A major advantage of the symbiotic relationship between the microbiota and the host, is the ability of these organisms to provide energy to the intestinal epithelium in the form of SCFA from fibrous sources which is otherwise not digestible to the animal (McDonald, 2011, Fouhse et al. 2016). It is important to keep in mind that a single layer of cells provide a barrier between the host and external pathogens and to maintain this barrier, the epithelial layer must be regenerated. The interaction between the epithelial layer and

microorganisms present in the GIT impacts the cell replacement rate and thus growth efficiency (Willing & Van Kessel, 2007).

In order to use less therapeutic antibiotics post-weaning, it is important to understand the host-microbial relationship in order to apply strategies to improve animal health and reduce antibiotic use. These strategies include the use of prebiotics, highly fermentable carbohydrate grains, probiotics or direct fed microbials and microbial transplants.

2.6 The use of *Bacillus*-based probiotics in pig nutrition

A DFM additive can have a number of positive effects on the host and can achieve this through a number of mechanisms, which include competition of nutrients, production of antimicrobial substances, host immunomodulation, intestinal adhesion and competitive exclusion (Fuller, 2004). Direct fed microbials available as additives to animal production are normally categorised in three main groups namely, *Bacillus* spp., lactic acid producing bacteria (LAB) and yeasts (NRC, 2012). Until recently, LAB from the genus *Lactobacillus* and *Pediococcus* were most commonly used in animal feeds as DFM. These organisms require refrigeration or lyophilisation in order to survive extended storage periods required by the animal feed industry as well as microencapsulation to withstand pelleting temperatures and feed application, therefore adding to the cost of in-feed application (Latorre *et al.*, 2016).

Increasing interest has been shown in the use of *Bacillus* spp. spores over Bifido- and Lactobacilli as DFM feed additive in monogastric animals; this is mostly due to their outstanding resistance to harsh conditions like pelleting and low pH, having a long shelf life, formation of biofilms, extracellular enzyme production and the secretion of antimicrobial substances (Hong *et al.*, 2005; Cutting, 2011; Larsen *et al.*, 2014; Latorre *et al.*, 2016). Organisms belonging to the *Bacillus* genus are gram positive, rod shaped and typically inhabitants of the soil. Spores of these organisms have proved to be able to persist, germinate, survive and reproduce in the GIT of different animal species, which advocates that these organisms could be considered as anaerobic bacteria and metabolically active among host microbiota (Cho *et al.*, 2011; Latorre *et al.*, 2014, 2016). A pH of 6-7 is optimal for *Bacillus* spores to germinate, grow and produce enzymes; germination begins in the nutrient-rich environment of the small intestine (Setlow, 2014). Therefore, from a commercial perspective, the use of a *Bacillus* spp. probiotic seems like a viable alternative to traditional DFM additives.

A comprehensive meta-analysis done by Zimmermann *et al.* (2016) indicated that DFM supplementation in pigs provided an improvement in production performance and health status. Production benefits resulted in an increase of average daily gain (ADG) by 29.9 g/ day (summarised from 32 studies with 67 experiments and 4,122 pigs) and the feed conversion ratio (FCR) by -96 g feed per kilogram live weight gain (summarised from

29 studies with 60 experiments and 4,011 pigs). The positive effects of probiotic supplementation was most evident during the first phase of rearing and the finishing phase in terms of ADG. The other benefit in terms of FCR was observed from wean to finishing phases (Zimmermann *et al.*, 2016). *Bacillus subtilis* proved to have an influence on nutrient digestibility, which could be one of the reasons for improved animal performance (Giang *et al.*, 2012). Increased animal performance could be explained by an increase in villi thickness and surface area when diets supplemented with *Bacillus* spp. were fed (Al-Baadani *et al.*, 2016).

A study conducted by Alexopoulos *et al.* (2004) investigated the efficacy of a dual strain *Bacillus* probiotic containing *B. subtilis* and *B. licheniformis* on the health status and performance of sows and their litters. This study found that the DFM improved certain blood and milk composition parameters in the lactating sow, which subsequently improved weight gain and the overall health status of nursery piglets up until the weaning period. The use of this particular probiotic increased sow feed intake during the first 14 days of lactation and a decrease in sow weight loss during the lactation period. The use of probiotics in the sow diet during lactation gave the piglets an indirect advantage by way of better milk quality and a farrow pen environment that was less contaminated by pathogenic bacteria originating from the sow (Alexopoulos *et al.*, 2004). The use of DFMs in the farrowing pen could have a positive influence on the colonisation of beneficial microbiota in the neonatal piglet's GIT, as well as the quality of the sow milk.

There is no doubt that the early weaning of piglets between 21 and 28 days of age is the cause of a range of GIT disturbances resulting in large economic losses in the pig industry. The weaning transition is a difficult and complex period when the piglets are forced to cope with the sudden separation from the sow, while being mixed with other litters in a new environment, as well as a change in the diet from highly digestible nutritious milk to a less digestible and more complex solid feed. These factors induce a shift in the beneficial gut microbiota to favour pathogenic and opportunistic organisms, which can cause PWD and its subsequent effects (Konstantinov *et al.*, 2006). Through the promising mechanism of competitive exclusion (among others) the addition of a probiotic could have a positive influence on the gut microbiota balance, intestinal epithelium integrity, appropriate development of the gut tissue and the functioning of the neuro-endocrine system (Metzler *et al.*, 2005). A DFM is expected to deliver at least one of a number of functions to the GIT in order to alleviate the effects of PWD in weaned pigs. These functions can briefly be described as firstly, stimulating the development of a beneficial and healthy gut microbiota environment, secondly preventing colonisation of enteric pathogenic bacteria, thirdly increasing the digestive capacity and the lowering of the gut pH, fourthly improvement of mucosal immunity and lastly, enhancement of gut tissue maturation and integrity (De Lange *et al.*, 2010).

A review by Gaggìa *et al.* (2010) indicated that the inclusion of a *B. subtilis* probiotic, reduced the incidence of scouring 24 hours after a challenge of K88-positive enterotoxigenic *E. coli* (ETEC) in weaned

piglets. The addition of a *B. subtilis* and *B. licheniformis*-based DFM to the diet of weaned piglets showed positive growth response in 30 of 31 performance studies (De Lange *et al.*, 2010). Alexopoulos *et al.* (2004) found that the administration of spores consisting of *Bacillus licheniformis* and *B. subtilis* reduced weaned piglet morbidity as well as mortality, subsequently improving performance parameters in the fattening period. The same *Bacillus*-based complex was effectively used in a high performing commercial setting to substitute AGPs without a decrease in weaned pig performance (FAO, 2016). The same author also reported that the supplementation of weaned piglet diets with *B. licheniformis* at certain dose rates, significantly reduced PWD and subsequent piglet mortality. The use of *Bacillus*-based probiotic also resulted in improved growth rates and feed efficiency in weaned piglets (Alexopoulos *et al.*, 2004; Cho *et al.*, 2011; Lan *et al.*, 2017). A study conducted by Jaworski *et al.* (2017) found that diets supplemented a DFM resulted in a better FCR of weaned pigs 0-14 days post-weaning compared to pigs that did not receive a DFM supplement, while ADG and ADFI were not affected significantly. Jørgensen *et al.* (2016) reported that the supplementation of a *B. subtilis* and *B. licheniformis* probiotic complex had significant growth rate effects on the entire post-weaning period of 28-70 days of age and improved growth by 6.1%.

Various studies have investigated the effects of *Bacillus*-based DFM on growing pigs and although improved growth rate and feed efficiency were found in some studies (Meng *et al.*, 2010), other studies showed only an improvement in grower feed efficiency (Davis *et al.*, 2008) or an improvement in feed intake and growth (Wang *et al.*, 2009; Kim *et al.*, 2014). Some studies indicated only improvement in growth rates for growing (Chen *et al*., 2005) and finishing pigs (Chen *et al.*, 2006). In a previously mentioned wean-to-finish study by Jørgensen *et al.* (2016), a *Bacillus*-based DFM additive significantly improved growth rate and FCR by 11.2% and 8.6%, respectively, in the grower period (70-120 days age). The subsequent finisher period (120-182 days of age) indicated significantly better FCR and significantly improved ADG and FCR over the wean-to-finish period of 28-182 days of age (Jørgensen *et al.*, 2016). It is evident that trial results in the use of *Bacillus*-based probiotics are highly inconsistent on performance in practise; this could be due to different diet formulations, raw feed ingredients used, differences in DFM strains, dose levels, age of animals supplemented with DFM additives and interactions with environmental factors (Jørgensen *et al.*, 2016). It is therefore important to evaluate the effects of DFMs over a longer period of time. It is also suggested that the nutrient density of diets could influence the performance effect and end result of probiotics in growing pigs (Meng *et al.*, 2010).

A multi-strain probiotic could potentially be more effective than mono-strain probiotic due to the specificity of the various single strains. Multi-strains could also provide more consistent results due to the complementary effects between different strains of species (Timmerman *et al.*, 2005). The effect and outcome of DFM supplementation and the outcome of their efficacy in the host animal, will most likely depend on the dietary ingredient composition (Merrifield *et al.*, 2013). New screening techniques, as well as genetic selection

of new and specialised organisms make it increasingly difficult to compare older data of probiotic organisms in terms of probiotic performance as the newer generation organisms tend to perform better and more consistently (Jers *et al.*, 2017).

2.7 The modes of action of direct fed probiotic organisms

Different probiotic organisms or DFMs exert their effects through a range of diverse mechanisms including known and unknown methods. Brown (2011) compiled a list of characteristics of a good probiotic and these characteristics include the following:

- Acid and bile resistance
- Strain specific and high ability to rapidly multiply in the gut
- Absence of any pathogenicity or toxicity to the host
- Strong adhesive capability within the digestive tract of the host
- Durability to withstand the duress of commercial manufacturing, processing and distribution
- Ability to reduce the pathogenic microorganisms

Unlike antibiotics, probiotics aim to improve overall health by an increased number of beneficial microbes colonising the gut. Strains within the same sub-species could differ in their mode of action and a summary of these various modes of action will be discussed briefly in the following sections.

2.7.1 Modulation of the gut microbiota

In order to maintain a healthy GIT environment, especially in the context of a reduction in sub-therapeutic antibiotic use, manipulation of the dietary raw materials and the use of additives like DFMs are of crucial importance to maintain and improve the performance of production animals (Gaggìa *et al.*, 2010). Probiotics are believed to improve the overall health status of animals by modifying the gut microbial population and preventing an imbalance between pathogenic and beneficial bacteria (Zimmermann *et al.*, 2016; Liao & Nyachoti, 2017; Liu *et al.*, 2018). A shift towards a beneficial composition changes the dynamics of the GIT, resulting in more efficient digestion and improved immunity (Liao & Nyachoti, 2017). There are two main mechanisms involved in the modulation of gut microbiota, namely competitive exclusion and direct antimicrobial inhibition.

Ccompetitive exclusion can be defined as the ability of normal intestinal microflora to protect against the establishment of harmful pathogens that increase the risk of intestinal infections and disorders in pigs (Cho *et al.*, 2011). This concept involves bacteria cultures of unharmful nature to compete with pathogenic bacteria for adhesion sites to the gut as well as nutrients or organic matter within the gut (Cho *et al.*, 2011). If these

beneficial cultures could adhere to the gut wall, it could prevent the colonisation of pathogenic and opportunistic bacteria on the gut wall (Brown, 2011; Liao & Nyachoti, 2017). The addition of a probiotic to the diet of weaning pigs was able to increase the counts of beneficial lactic acid producing bacteria, thereby enabling the GIT to become more competitive or antagonistic against harmful organisms and decrease pathogenic *Clostridium* spp., *E.coli* and *Enterobacterium* spp. (FAO, 2016). According to the same author, pathogenic bacteria need to attach to the GIT wall in order to exert their pathogenic effects on the host. Therefore, an expected effect of adding DFMs to the diet is that an increased amount of beneficial bacteria could cause an inhibition in the adhesion of pathogenic bacteria on the gut wall (Liao & Nyachoti, 2017). Oelschlaeger (2010) reported that it was possible to reduce adhesion of pathogenic *E.coli*, *Clostridium*, and *Salmonella* strains to the intestinal mucus layer of the pig with an inclusion of a DFM. The competitive exclusion effect of DFMs is also brought along by the fact that these organisms compete for nutrients, as well as nutrient absorption sites in the GIT (Liao & Nyachoti, 2017). The competition between the probiotic organism and other bacteria for energy and other nutrients could lead to a decrease in the growth rate of the pathogenic bacteria due a reduction in the readily available nutrients within the digesta (gram positive and gram negative) and are able to shift the microbiome status to a beneficial status (Cho *et al.*, 2011; Liao & Nyachoti, 2017). Reproduction of the DFM is important, as the probiotic organism must be able to colonise the gut in order to sustain competitive exclusion. Studies have shown that a DFM of *B. subtilis* origin was able to persist and sporulate for up to 36 days in the avian intestine after a single dose, these organisms were also found to persist longer in the mouse gut (Hong *et al.*, 2005).

Apart from competitive exclusion, modulation of gut microbiota is also caused by direct antimicrobial inhibition (Latorre *et al.*, 2016). Most probiotic bacteria have the ability to produce SCFAs (such as lactic and acetic acids) through carbohydrate fermentation of complex polysaccharides. This creates a drop in the intestinal lumen pH to such an extent that it becomes difficult for harmful bacteria to tolerate, which further leads to the inhibition of cell wall synthesis and the formation of pores within the bacterial cell membrane, leading to death of the bacterium (FAO, 2016; Liao & Nyachoti 2017). Death of sensitive bacteria is caused by the drop in intestinal pH levels due to the production of substances like SCFAs by probiotic organisms that reduce intracellular pH levels of some pathogenic microbiota to fatal levels (Cho *et al.*, 2011; FAO, 2016). The production of substances that decrease the gut pH may offset the lower quantity of hydrochloric acid secreted in the stomach of piglets which could also aid digestion (Liao & Nyachoti, 2017). Other substances produced by probiotic organisms include hydrogen peroxide from *Lactobacillus lactis*, which inhibited the growth of *E. coli* on refrigerated chicken meat. Furthermore, *Bacillus* organisms like *B. subtilis* and *B. amyloliquefaciens* inhibited the growth of *Clostridium perfringens* which is the causative agent of necrotic enteritis in broilers, while the latter probiotic improved performance in broilers by production of a range of lipopeptide compounds and polyketides that inhibited the growth rate of pathogens (FAO, 2016).

Another potential mode of antimicrobial inhibition is the formation of biofilms. Biofilms are suggested to have a protective role in shielding the bacterial cells from antimicrobial substances, gastric juices and improve adhesion to the mucosal surface of these beneficial organisms (Larsen *et al.*, 2014). The same authors observed the biofilm production capacity of various *Bacillus* spp. strains and found that there was a positive correlation between the ability to sporulate quickly and biofilm production, with isolates of *B. amyloliquefaciens* subsp. *plantarum* and *B. licheniformis* being able to produce biofilms at moderate to high levels. Latorre *et al.* (2016) conducted a biofilm assay and results indicated that 11 of the 31 DFM species tested, synthesised a thicker and stronger adherent layer and indicating strong biofilm synthesis.

2.7.2 Modulation of host immune responses

The enterocytes of the intestine provide a selectively permeable barrier of defence that prevents the passive loss of nutrients, while blocking access of pathogens from the GIT to enter the host through the blood (Brown, 2011). The GIT is known as the first line of defence against pathogenic organisms and substances. This organ has a combined defence function that incorporates anatomical structures, secretions of immunological nature like mucus, immunoglobulins (IgA, IgG, IgM), antimicrobial peptides, as well as the epithelial junction adhesion complex, i.e. the tight junctions that seal adjacent epithelial cells of the GIT (Lee *et al.*, 2014; FAO, 2016). Disease conditions cause a disturbance in the immunological balance and disrupt the defence barrier of the GIT, prompting inflammation and intestinal disorders (FAO, 2016; Roselli *et al.*, 2017). Direct-fed microbials have the ability to affect the GIT component of the immune system from a range of antigens present in the lumen; this affects both the innate and adaptive immune system.

Direct-fed microbials enhance the innate immunity by preventing chronic inflammation of the GIT through stimulation of the innate immune system in the gastrointestinal epithelium (FAO, 2016). Some probiotic supplements are capable to act as immunomodulators that enhance the macrophage activity and increase local antibody levels to stimulate the production of interferon that activates killer cells (Cho *et al.*, 2011). The protective effects of a *Lactobacillus plantarum* DFM was investigated against the damages on the epithelial barrier induced by enterotoxigenic *E. coli* (ETEC) K88 in the differentiated epithelial cell line (IPEC- J2). It was found that the DFM weakened the *E. coli* upregulation of interleukin 8 (IL-8) as well as TNF-α gene expression, in other words, the cells of the epithelial barrier were protected against damage through sustaining the gene expression and the subsequent contents of the critical tight junction proteins (Wu *et al.*, 2016). Furthermore, a reduction in the translocation of ETEC to the mesenteric lymph nodes was noted in weaning piglets with the supplementation of a DFM (*P. acidilacti*) when compared to the control group after an ETEC challenge (FAO, 2016).

Stimulation or suppression of acquired immunity in humans and animals is crucial in certain circumstances, for example, the stimulation of the immune system is required in infection and immune

deficiency situations, while suppression of the immune system is necessary in allergy and autoimmune disease situations, all depending on the clinical conditions present (Liao & Nyachoti, 2017). The responses of DFMs on the immune system are complicated and it varies between different probiotic strains and species, the dose level of the DFM, the stress situation, i.e. pre- and post-weaning, and if the antigen is in the form of a bacterium or virus. Depending on the type of probiotic organism fed, it is possible to affect the expression of the anti-inflammatory cytokine or cell signalling protein, which will also differentiate between cytokines (FAO, 2016; Roselli *et al.*, 2017). It was reported that feeding a *Lactobacillus fermentum* DFM to piglets upregulated the pro-inflammatory cytokines, as well as the percentage of CD41 lymphocyte subset in the blood (Cho *et al.*, 2011). In another study, the addition of a *Bacillus subtilis* probiotic to broiler feed, increased the antibody response to sheep red blood cells administered to the test subjects (Afsharmanesh & Sadaghi, 2014). Interestingly, in a different instance a probiotic product was able to increase antibody titres against deadly poultry diseases like Newcastle Disease, Infectious Bronchitis and Infectious Bursal Disease (Landy & Kavyani, 2013). In a study done on weaner piglets, it was demonstrated that a probiotic (*B. cereus* var. *toyoi*) significantly increased the population of intra-epithelial CD8+ T-cells and that the number of gamma delta T-cells tended to be higher in the intestinal epithelium at 28-day weaning (Cho *et al.*, 2011). Increasing evidence indicates that the positive effect of DFMs on the immune system is associated with elevated humoral and cellular immune response through the increased production of T-lymphocytes, CD+ cells and antibody secreting cells, the expression of pro- and anti-inflammatory cytokines, interleukins, IFN-*y*, natural killer cells, antibody production, respiratory burst of macrophage cells and delayed hypersensitivity reaction (Brown, 2011).

2.7.3 Antitoxin effects

It is possible that toxins are one of the most important groups of bacterial virulence factors and the effectiveness of certain probiotic organisms to protect the host against diarrhoea is likely based on their ability to inhibit toxin expression by pathogenic organisms (Oelschlaeger, 2010). Compounds produced that hampers toxin expression by probiotic organisms include organic acids, antioxidants and bacteriocins. These compounds not only reduce the viable numbers of pathogenic organisms, but they may also affect bacterial metabolism and toxin production (Cho *et al.*, 2011).

Lactobacilli and certain *Bacillus* species have been reported to produce bacteriocins like acidophilin, lactocidin, lactolin and acidophilin. These substances have been demonstrated in *in vitro* studies to have an inhibitory action against a range of pathogenic bacteria like *Salmonella* spp. and *E. coli* (Brown, 2011). Bacteriocins produced by lactic acid bacteria have also been able to infiltrate the outer membrane of gram-negative bacteria and subsequently induce an inactivation of the cell in conjunction with factors like low temperatures, organic acids and detergents (Cho *et al.*, 2011). Various studies (Hong *et al.*, 2005, Lallès *et al.*, 2007b, Lallès *et al.*, 2007a and Liao & Nyachoti, 2017) have shown a reduction of diarrhoea in piglets that

have received probiotics, independent of the microorganisms used in the probiotic feed product. It is likely that the enterotoxins produced by pathogenic bacteria could be the cause for intestinal fluid loss and diarrhoea.

2.7.4 Modulation of nutrient digestibility

Supplementation of probiotics to animal diets is known to have positive effects on digestibility of nutrients like dry matter, organic matter, energy, crude protein, crude fibre and phosphorous and animal performance and are used world-wide in monogastric nutrition (Meng *et al.*, 2010; Zimmermann *et al.*, 2016; Lan *et al.*, 2017; Liao & Nyachoti, 2017). The increased digestibility of nutrients with DFM supplementation may be due to the increased enzyme activity in the intestine (Larsen *et al.*, 2014). *Lactobacillus* probiotics were able to alter the digestive enzyme activity in the GIT of pigs and broilers by increasing the amylase activity by 42% and subsequently increasing body weight gain and feed efficiency by 4.6% and 5%, respectively (FAO, 2016). The increased enzyme activity within the GIT of the host supplemented with a DFM could be due to either production of extracellular enzymes by the probiotic organism or an induced change in the microbial population within the gut and therefore a change in the host enzyme production. Improvements in total tract digestibility of nitrogen and energy were found in pigs supplemented with a 0.1% *L. reuteri* and *L. plantarum* probiotic product ($1x10^6$ CFU/g) at the end of a 4-week treatment phase (Upadhaya *et al.*, 2015).

The improvement in digestibility of dietary nutrients in monogastric animals may largely be a result of increased production of extracellular enzymes by probiotic organisms as they possess a high fermentative activity and can therefore enhance nutrient digestion along the GIT of the host (Cho *et al.*, 2011). Spore-forming bacteria (*Bacillus subtilis, B. licheniformis, B. amyloliquefaciens*) are known to produce extracellular enzymes that include α-amylase, cellulase, lipases, proteases and metalloproteases (Priest, 1977; Carlisle & Falkinham, 1989; Davis *et al.*, 2008; Larsen *et al.*, 2014; Zaghari *et al.*, 2015; Banerjee *et al.*, 2017). A study done by Larsen *et al.* (2014) indicated that the production of cellulase and xylanase varied significantly among and within the *Bacillus* species. Furthermore, isolates of *B. amyloliquefaciens, B. mojavensis* and *B. subtilis* produced significant amounts of both enzymes, resulting in a release of 50-70 mU/mL in the case of xylanase. Meng *et al.* (2010) reported that growing pigs fed a combination of *B. subtilis* and *Clostridium butyricum* DFM endospores had improved crude protein and energy digestibility when compared to non-treated pigs.

The use of probiotics increased nutrient absorption capacity of the intestinal mucosa by improvement of the height of the villi as well as the villus height: crypt depth ratio, thus increasing surface area for nutrient absorption (Lee *et al.*, 2014; FAO, 2016). A DFM consisting of one strain *B. subtilis* and two strains *B. amyloliquefaciens* were able to increase intestinal villus height as well as the length of the duodenum, jejunum and ilium in post-weaning pigs (Liao & Nyachoti, 2017). In a different example, the villus height in *Bacillus* probiotic treated broilers was greater than in birds that were treated with an AGP (zinc bacitracin) at six weeks of age (Hung *et al.*, 2012). In a similar study, a *B. subtilis* probiotic was able to reconstruct the normal structure

of necrotic enteritis damaged intestinal villi that were caused by *Clostridium perfringens* (Jayaraman *et al.*, 2013).

2.7.5 Other modes of action

Although the exact methods are not well understood currently, other modes of action of DFMs are being investigated; these include antioxidative activity and alleviation of stress and altering bacterial and host gene expression.

Pigs housed in modern day industrial farming systems are exposed to various factors that can lead to oxidative stress, as a result impairing the host immune system and this may cause chronic diseases due to oxidative damage. Certain lactic acid bacteria in particular *Bifidobacterium longum* and *Lactobacillus fermentum* were able to produce antioxidants and scavenging free radicals *in vitro.* This could thus be used as a strategy to lessen the effects of oxidative stress. In another study, the antioxidant status of pigs in the growing to finishing stages was improved by supplementation of *L. fermentum* as increased serum levels of antioxidant enzymes (i.e. superoxide dismutase and glutathione peroxidase) as well as decreased serum and muscle levels of malondialdehyde were recorded (Liao & Nyachoti, 2017).

The alteration of bacterial and host gene expression could also be possible with the use of DFM organisms. Bacterial communities are able to communicate through secreted chemical signals known as auto-inducers that are produced as a result of changes in the cell population density. This bacterial communication process affects cell behaviour of the bacteria, as well as of the host, and is called quorum sensing (Hughes & Sperandio, 2008). A probiotic organism was able to affect *in vitro* quorum sensing and influence the pathogenicity of human enterohaemorrhagic *E. coli* O157:H7. This was done as a result of fermentation products of *L. acidophilus* that inhibited the chemical signal (autoinducer-2) of the pathogenic *E. coli*, resulting in a suppression of the virulence gene, thereby disrupting quorum sensing and colonisation in the host GIT (Medellin-Peña *et al.*, 2007).

In conclusion, the modes of action of DFMs are not yet fully understood and more research is needed in order to explain the functions of the different probiotic organisms as well as the interactions between DFMs, pathogens and the host (Liao & Nyachoti, 2017). In order to explain the beneficial effects and modes of action of probiotics, a clear understanding of the direct and indirect mechanisms are required. It is possible that the positive effects of probiotic supplementation in animals reported could be due to a combination of the various mechanisms or actions.

2.8 Enzyme production of *Bacillus* organisms

Bacillus spp. are known to be the dominant bacterial workhorses in industrial fermentation and widely used for the large scale production of extracellular enzymes as well as proteins (Schallmey *et al.*, 2004; Degering *et al.*, 2010). *Bacillus* organisms have been an attractive species for use in industrial fermentations for a number of reasons, including rapid growth rates, short fermentation cycle times, their capacity to secrete proteins in the extracellular medium as well as generally recognised as safe (GRAS) status in the USA (Schallmey *et al.*, 2004). Species belonging to the genus *Bacillus* have a vast amount of members that are bigger in numbers than that of the genus *Lactobacillus*, and therefore a larger genome capacity for the selection of encoding enzymes (Fogel *et al.*, 1999). It is estimated that enzymes originating from Bacilli make up about 50% of the total fermented enzyme market, which is made up of food enzymes (29%), feed enzymes (15%) and general technical enzymes (56%) (Schallmey *et al.*, 2004).

In animal production, the addition of DFM organisms may improve digestion of the NSP content of pig feeds, by the microbial enzymes produced throughout the gut from the microorganisms present (Upadhaya *et al.*, 2015). Latorre *et al.* (2016) investigated the *in vitro* enzyme activity of 31 screened Bacilli species strains and found that enzyme activity was detected in the majority of the strains; there were also considerible differences in the relative enzyme activity between strains of the same species. Differences were found in the type of enzymes secreted among species and strains, for example some isolates were excellent amylase producers, while other strains produced more protease, phytase or lipase than others. Various independent studies have shown that Bacilli are able to produce a range of enzymes which include fibre degrading enzymes like cellulase (Hendricks *et al.*, 1995), β-glucanase (Aono, 1992), α-amylase (Elamin Ibrahim *et al.*, 2012), α-galactosidase (Talbot & Sygusch, 1990), β-mannanase (Talbot & Sygusch, 1990), xylanase (Monisha *et al.*, 2009) as well as other enzymes that include protease (Carlisle & Falkinham, 1989; Banerjee *et al.*, 2017), lipase (Shah, 2012), keratinase (Mazotto *et al.*, 2011) and phytase (Choi *et al.*, 2001). Results of a particular study (Latorre *et al.*, 2016) indictated that differences exist in the enzyme producing capacity of Bacilli strains within the same species, futhermore the type of enzyme produced differs between organisms and is strain specific. Wang & Gu (2010) tested different levels of *B. coagalans* supplementation in broilers and found increased protease and amylase activity at a lower inclusion rate of $2x10^6$ CFU/g; this subsequently led to improved digestion of protein and starch. The concluding remarks of this particular study was that different *Bacillus* strains fed to broilers were able to produce a wide range of bacterial enzymes and/or stimulate endogenous production of enzymes. In another study, a *B. subtilis* DFM was isolated from the GIT of a certain fish species and showed a promising production of amylase (38.23 ± 1.15 µg of maltose liberated mg^{-1} protein mL^{-1} of culture filtrate) followed by cellulase (23.1 ± 2.4 µg of glucose liberated mg^{-1} protein mL^{-1} of culture filtrate) and protease (9.2 ± .08 µg of liberated mg^{-1} protein mL^{-1} of culture filtrate) (Banerjee *et al.*, 2017). Enzymes produced by certain Bacilli demonstrated exceptionally high levels of enzyme production by producing 20-25 g / litre of a specific enzyme (Schallmey *et al.*, 2004). Larsen *et al.* (2014) found that a

combination DFM consisting of *B. subtilis* and *B. licheniformis* produced glycosyl enzymes, which assisted in the hydrolysis of glycosidic bonds in complex sugars. The same DFM compound was able to produce α-amylase and protease extracellularly in a separate study, which could also enhance nutrient digestibility and subsequently growth performance (Carlisle & Falkinham, 1989). Figure 2.1 summarises the amount of cellulase and xylanase enzymes produced by a range of DFMs and strains after two independent experiments (Larsen *et al.*, 2014). Depending on the strain of organism, it might be possible to acquire DFMs that are able to produce specific amino acids *in situ*; this could be important to the producer to alleviate the pressures of adding synthetic amino acids in the diets of pigs (Nørgaard *et al.*, 2016a). However, it does not seem that a specific *Bacillus* probiotic is able to significantly affect the apparent ileal digestibility and standardised ileal digestibility of crude protein and amino acids in growing pigs (Kaewtapee *et al.*, 2017).

The synthesis of extracellular enzymes by probiotic organisms could lead to an improvement in the feed efficiency, reduced diet costs and increased utilisation of cereal by-products. It is, however, important to note that the correct strain of DFMs should be selected for the application of enzyme secretion as there exists a large variation in the types and amounts of exogenous enzymes produced between different organisms and strains.

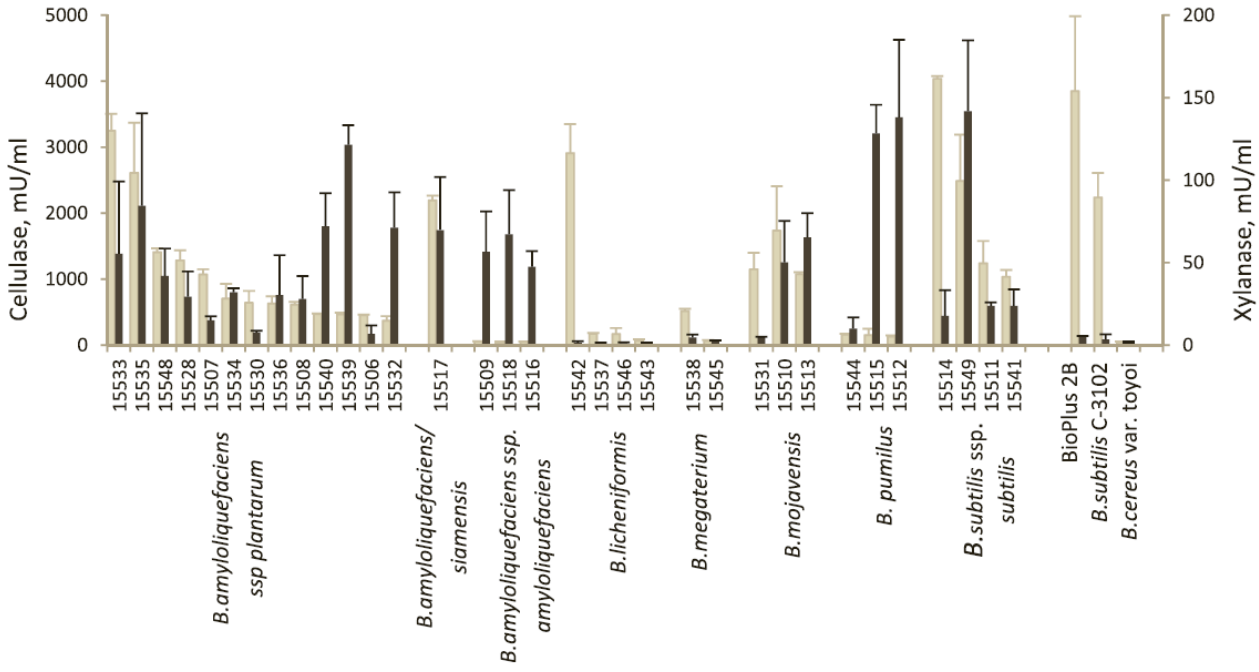

Figure 2.1. The production of cellulase (light bar) and xylanase (dark bar) of different *Bacillus*-based probiotic organisms and subsequent strains (Larsen *et al.*, 2014)

2.9 The utilisation of non-starch polysaccharides by probiotic organisms

The NSP fraction of modern pig diets is composed of different combinations of monosaccharides to form complex structures that make up oligometric and polymetric structures. Variation in structure complexity between different fibre sources will affect the gut microbiota, as these organisms have certain and specific bonding sites on complex fibre molecules for microbial degradation and fermentation (Hamaker & Tuncil, 2014). Microbial enzyme degradation in the small intestine alter the structure and size of the NSP molecule; this enables microbial fermentation in the large intestine and facilitates further degrading of partially degraded NSP, thus increasing nutrient digestibility and causing a prebiotic effect in the large intestine (Bach Knudsen *et al.*, 2016). An increase in the efficacy of NSP and dietary fibre particle breakdown could later act as prebiotic substances and stimulate proliferation of beneficial cellulolytic bacteria in the lower GIT (Metzler *et al.*, 2005; Jers *et al.*, 2017; Roselli *et al.*, 2017).

A study conducted by Merrifield *et al.* (2013) indicated that diet composition plays a key role in the effect a DFM ultimately has on the host. In this specific study, different weaning diets induced divergent and sustained shifts in the metabolic phenotype of weaning piglets. When *B. lactis* supplemented diets were fed, the systematic metabolism was affected throughout the different diet groups over and above the effects of the diets. Wealleans *et al.* (2017) investigated the effects of different feed additives with and without the addition of a 3-strain *Bacillus* DFM in broiler chickens. Results of this study indicated a clear additive benefit of using the particular probiotic complex together with other extracellular enzymes like xylanase and amylase complex (XA), as well as a xylanase, amylase and protease complex (XAP) in terms of apparent ileal digestibility of energy. The authors hypothesised that the additional energy released came from the fibre fraction of the diet as a result of the synergistic combination of the DFM and additional enzymes. Wealleans *et al.* (2017) also investigated the degree of NSP hydrolysis by measuring the reduction in the particular NSP molecules in the dry matter fraction. The DFM and enzyme combinations led to a reduction in the flow of insoluble arabinose and galactose on ileal and total tract levels when compared to control broiler birds. On total tract digestibility, a reduction in the soluble and insoluble fraction of xylose was recorded in the case of the DFM and XAP combination treatment.

Research conducted by Davis *et al.* (2008) found that a *Bacillus*-based probiotic (two strains of *B. licheniformis* and one strain of *B. subtilis*) was able to increase the dietary energy content by enhancing dietary fibre fermentation in growing-finishing pigs. A study by Lan *et al.* (2017) investigated the interactive effects of a *Bacillus*-based DFM and dietary nutrient density in growing pigs that had a difference of 50 kcal ME/kg and 10 g/kg crude protein difference between the high and low density diets. Results indicated that pigs fed high and low density diets supplemented with a DFM, were able to compensate for lower energy with increased feed intake where lower energy diets were provided in the 0-42 day period after weaning. In the same study, the more nutrient dense diet had significant higher digestibility of dry matter, nitrogen and gross energy when

compared to the pigs offered the lower density diets. The added probiotic complex did, however, significantly improve gross energy digestibility. Another study found that probiotic efficiency was better where higher energy and protein specification diets were fed when compared to lower density diets (Meng *et al.*, 2010). In his conclusion, Lan *et al.* (2017) indicated that the beneficial effects of DFM supplementation on the average daily feed intake of pigs were more dramatic in diets lower in nutrient density.

Increasing the dietary fibre content in broiler diets in conjunction with a DFM did not improve ADG and ADFI in the particular study of Jaworski *et al.* (2017) and resulted in a lower BW at 43 days of age, without affecting the FCR of the animals in the study. Furthermore, the addition of the DFM had no effect on pH or the VFA concentrations in ileal, caecal or rectal contents, even though differences in concentration of acetate, propionate, isovalerate, total short chain fatty acids, and total branched-chain fatty acids were recorded in the rectal contents of low fibre diets when compared to high fibre diets. Interestingly, an earlier study by the same authors indicated that a similar *Bacillus* DFM added to growing pig diets containing different sources of DF was able to increase fibre fermentation and subsequently, available metabolisable energy (ME). An increased concentration of VFAs was recorded in the faeces of the DFM supplemented diets and resulted in an increase in the ADG and final BW of the test animals (Jaworski *et al*., 2014a).

Research by Jørgensen *et al.* (2016) indicated that the composition of pig diets influenced the activity of probiotic organisms. In this study, a total of 576 pigs from wean to finish received either a standard diet or a diet containing 3% less net energy. Using a 2x2 factorial trial arrangement, the effect of a DFM containing *B. subtilis* and *B. licheniformis* were tested. Results indicated that there was an interaction ($P<0.05$) between the probiotic and dietary energy level during the grower period. Pigs that received the lower energy diet with the probiotic included, had significant improved BW, ADG and FCR than the same energy density without the probiotic. Moreover, the final BW, ADG, and FCR of pigs fed the reduced energy diet with the probiotic complex, did not differ significantly from those animals fed the standard energy diet without the DFM complex.

2.10 Conclusion

In conclusion, the use of dietary ingredients containing high levels of NSP can affect the feed efficiency and performance of pigs. Without the use of appropriate feed additives to reduce dietary viscosity, the NSP content will most probably have negative effects on animal performance. Early hydrolysis of dietary polysaccharides may change the structure of dietary fibre that migrates to the large intestine. This could have a positive effect in the large intestine by enhancing fibre fermentation efficiency and production of beneficial fermentation products. Additionally, shifting the microbiota to a beneficial cellulolytic population in a low pH environment helps to prohibit migration of proteolytic bacteria up the GIT of the growing pig. The various

modes of action of DFM organisms make these additives a viable alternative to antibiotics. The exact mechanism of these organisms differ between strains of the same species and make it very difficult to predict an exact response. The variation between results of studies could be due to a range of factors that includes but is not limited to the dietary fibre type, formulation, dietary nutrient specification and generation of DFM organism as well as species/strain of DFM used. The environment, including various stressors such as ambient temperature, stocking density and hygiene, may also influence results. Indications that DFM supplementations are able to utilise the dietary fibre fraction by means of extracellular enzyme synthesis, could aid the host in terms of available nutrients and increase feed efficiency apart from the possible health benefits.

CHAPTER 3

MATERIALS AND METHODS

3.1 Trial design and treatments

In order to evaluate the effects of a commercially available probiotic on the dietary energy content and performance of wean to finish pigs, a study was conducted using a completely randomised block design. The randomised block design consisted of 14 blocks, each block consisted of one of each of the four different treatments. Treatments within blocks were assigned to two treatments (standard energy and reduced energy) and two subsequent levels (with probiotic and without probiotic). The trial was conducted on the Hillcrest Experimental farm, University of Pretoria (Pretoria, South Africa) under controlled conditions. Pigs were randomly allotted to 1 of 4 treatments in a 2x2 factorial arrangement of treatments. Five feeding phases were used to feed 168 male pigs over an 18-week (126 days) trial period.

The four treatments used in this trial are described in table 3.1. Treatments 1 and 2 contained a standard energy content often used in commercial pork production, while Treatments 3 and 4 contained a fixed reduced energy content. Pigs in Treatment 1 were fed a standard energy diet supplemented with 400 mg/kg of Bioplus YC probiotic (Chr. Hansen, Denmark), while animals in Treatment 2 were fed the standard energy diet without any supplementation of the probiotic. Pigs allocated into Treatment 3 were fed the reduced energy diet supplemented with 400 mg/kg of Bioplus YC probiotic, while animals in Treatment 4 were offered the same reduced energy diet without the supplementation of the probiotic. All treatments in the trial had a total of three pigs per pen and 14 pen replicates per treatment.

Table 3.1. Treatment diets used in the trial to evaluate the effect of Bioplus YC probiotic on the energy availability of growing pig diets

Treatment	Energy density	Bioplus YC*	Replications
Treatment 1	Standard energy	400 g/ton	14
Treatment 2	Standard energy	0	14
Treatment 3	Reduced energy	400 g/ton	14
Treatment 4	Reduced energy	0	14

*Bioplus YC is a dual strain probiotic product consisting of *Bacillus subtilis* and *Bacillus licheniformis* that is produced by Chr. Hansen (Denmark) with a recommended dosage rate of 400 g/ton for growing pigs

Formulation of experimental diets was done on a least cost basis using least cost formulation software (Spesfeed feed formulation software, Spesfeed, South Africa) to formulate the five phase diets. The

composition of the trial diets is shown in table 3.2. Four treatments were fed during each phase which included two standard energy diets (Treatment 1 and 2) and two reduced energy diets (Treatment 3 and 4). The lower energy diets (Treatment 3 and Treatment 4) were reduced by 0.3 MJ/kg or 72 kcal/kg on net energy level (NE). This net energy reduction was applied to all of the reduced energy diets throughout the trial. Treatments 1 and 3 received 400 mg/kg Bioplus YC (Chr. Hansen, Denmark) probiotic throughout the whole trial. Treatments 2 and 4 did not receive any Bioplus YC; instead a 400 mg/kg inert carrier was added. Bioplus YC is a zootechnical additive that is manufactured by Chr. Hansen in Denmark. Bioplus YC is a dual strain spore-forming probiotic, consisting of *Bacillus subtilis* and *Bacillus licheniformis* and contains a minimum CFU count of $3.2x10^9$/g of Bioplus YC product. The recommended use for pigs in general is 400 g/ton of final feed.

Representative samples of maize and soybean meal were obtained and analysed prior to feed formulation in order to formulate the trial diets based on the exact nutrient profiles of the two main raw materials used. The proximate analysis was conducted according to the Association of Analytical Chemists (AOAC international) official methods of analysis as described in section 3.5.

A phytase enzyme, Axtra Phy 10 000 TPT (Du Pont- Delaware, United States of America) was included in all trial diets at 100 mg/kg to provide 1000 FTU/kg. The phytase enzyme's matrix values were included in the formulation according to the manufacturer's recommendations. A premix containing vitamins, minerals, choline and a standard mycotoxin binder (Freetox, Nutrex, Belgium) was added to all trial diets at 3 kg/ton. No antibiotic growth promotors were used in the trial. Zinc oxide was added to the creep diets at 3 kg/ton to all creep treatments. All trial feeds were formulated in such a manner that the diets were still comparable to commercial diets used in the South African pig industry.

Mixing of the trial diets was done at Simple Grow Agricultural Solutions (Knoppieslaagte, Centurion, Pretoria, South Africa), using a fountain feed blender. To avoid cross contamination of the probiotic, the total required amount of a specific feeding phase in both energy densities was first produced without probiotic (standard energy and reduced energy mash feeds). The two basal feeds (standard and reduced energy) within a dietary phase was manufactured with the same batch of raw materials to limit variation caused by raw material quality. The two basal groups of mash feed were each divided into two equal sub-groups. The one subgroup was directly pelleted, while the other subgroup was reblended with the addition of the probiotic product and pelleted after reblending. This process was repeated for both the energy levels and all five feeding phases. Final feed samples of all phases from the two treatments containing probiotic supplement were sent to CHR. Hansen's (Denmark) analysis laboratory to determine the CFU spore count. A variation of 30% was regarded as acceptable according to CHR. Hansen's standards.

Table 3.2. Feed ingredient and calculated nutrient composition of trial diets for standard energy (SE) and reduced energy (RE) in various feed phases

	Creep		Weaner		Grower 1		Grower 2		Finisher	
	SE	**RE**	**SE**	**RE**	**SE**	**RE**	**SE**	**RE**	**SE**	**RE**
Ingredients (%)										
Maize (yellow)	46.04	48.31	61.57	56.07	62.83	57.33	63.72	57.42	63.36	57.08
Soybean Oilcake (46%)	24.00	24.00	19.50	19.50	26.00	26.00	25.00	25.00	22.00	22.00
Full Fat Soybean	9.67	2.22	9.63	8.96	2.13	1.46				
Wheat Bran	2.00	6.83	2.00	8.26	5.67	11.92	8.59	15.00	11.44	17.83
Brewer's Yeast	2.00	2.00								
Dextrose	1.50	1.50								
Fish Meal (63%)	2.00	2.00	4.30	4.30						
Whey Powder	10.00	10.00								
Limestone	0.82	0.83	0.76	0.78	1.02	1.04	1.12	1.14	1.09	1.11
Monocalcium Phosphate	0.02	0.02	0.54	0.48	0.36	0.29	0.29	0.22	0.29	0.22
Salt	0.41	0.40	0.50	0.49	0.62	0.61	0.62	0.61	0.62	0.61
Zinc Oxide	0.30	0.30								
L-Threonine	0.11	0.18	0.18	0.18	0.18	0.18	0.04	0.03	0.21	0.20
L-Tryptophan	0.04	0.06	0.07	0.06	0.06	0.05	0.01	0.00	0.04	0.03
L-Valine	0.03	0.10	0.08	0.08	0.07	0.06	0.00	0.00	0.02	0.00
Lysine HCL	0.33	0.48	0.46	0.45	0.46	0.46	0.18	0.16	0.37	0.35
Methionine Hydroxy Analogue*	0.25	0.29	0.25	0.25	0.20	0.19	0.03	0.02	0.14	0.13
Vitamin and mineral premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Axtra Phy 10000 TPT**	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
BioPlus YC	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Inert Carrier	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
Mycotoxin Binder	0.1	0.1	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Additives***	0.025	0.025	0.025	0.025					0.03	0.03
Calculated nutrient composition (%)										
Dry Matter	90.08	89.99	89.15	89.05	89.02	88.93	88.85	88.76	88.21	88.69
DE Pigs (MJ/kg)	14.69	14.27	14.46	14.08	14.19	13.81	13.93	13.55	13.74	13.36
NE Pigs (MJ/kg)	10.35	10.05	10.21	9.91	9.96	9.66	9.79	9.49	9.74	9.44
Crude Protein	22.75	21.26	21.24	21.56	19.58	19.90	18.38	18.89	17.74	18.24
Crude Fibre	3.34	3.59	3.56	4.02	3.97	4.44	4.17	4.65	4.31	4.79
Fat	4.55	3.49	5.02	4.94	3.58	3.51	3.33	3.35	3.36	3.39
Calcium	0.7	0.7	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Total Phosphorus	0.67	0.67	0.62	0.66	0.67	0.72	0.68	0.73	0.70	0.75
Sodium	0.3	0.3	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine (Total)	1.55	1.55	1.51	1.51	1.36	1.36	1.08	1.09	1.16	1.17

* MHA, NOVUS International, USA

** Axtra Phy 10 000 TPT, Du Pont, Delaware, USA

*** Non-nutritive sweeters and Ractopamine

3.2 Experimental animals

One hundred and seventy four male pigs from the PIC 337 line (Pig Improvement Company, USA) with an average body weight of 6.81 kg ± 0.587 kg, were obtained from RK Farming (Bela-Bela, South Africa). The piglets were randomly selected on weaning day from that week's weaned piglet batch on 21-days of age. The selected piglets were transported and delivered early morning to the experimental farm's grower pig unit.

Piglets were immediately offloaded into holding pens prior to being divided into the trial house. During this time, piglets had no access to food and water to prevent inaccurate weight measurement of piglets. Piglets were quickly processed, which included individual tagging and weighing. After processing the piglets in the holding area, the individual piglets were divided into light, medium and heavy groups and classified in nine weight classes (LL, LM, LH, ML, MM, MH, HL, HM, HH). An equal amount of piglets from each of the nine weight groups were randomly allocated to the various treatments, divided evenly within the trial facility to minimise starting weight variation between treatments. Piglets of similar size were placed in a pen to limit bullying and dominance. Pens containing smaller and larger piglets were evenly spread out throughout treatments and the trial house to limit variance.

Upon arrival, all animals were in good health and injury free. Processing was conducted as fast and accurately as possible to limit stress and discomfort experienced by the piglets. After individual placement in the trial pens, piglets received *ad libitum* feed and water. Piglets were allowed a one week adaptation period before the commencement of the trial, during which they all received the same creep feed as before weaning.

3.3 Housing, environmental and feeding management

All animals were housed in an enclosed pig grower unit. Facilities used included 58 pens (3.5m^2 area in each pen) in an enclosed housing environment with extraction fan ventilation and automatic temperature control. The grower unit had glass windows on the northern and southern sides of the building. Fans on the northern side and air inlets on the southern side were installed to ensure adequate ventilation and removal of heat and gas from the grower unit. The in-house temperature was regulated automatically and a natural lighting program was followed throughout the trial. Ambient temperature was recorded daily. Three pigs were placed in each pen to ensure a stocking density of 1.166 m^2, which is comparable but still below the normal stocking densities practised in the South African pig industry.

Each pen had partially slatted concrete floors, one nipple drinker and one feed trough (CAVI International, Netherlands). During the creep phase, one rubber mat and an infrared heat lamp were provided per pen, as piglets arrived early spring and minimum temperatures were still relatively low. A diesel heater was also

installed to compensate for lower minimum temperatures. The infrared lamps provided ample heat during the creep phase. The automatic temperature and ventilation system was set to regulate the ambient temperature in a step down program as the piglets aged towards the weaner phase (PIC 337 Production Manual, USA). This system also regulated ambient temperature automatically to prohibit over and under heating within the trial house. Feed and water were available *ad libitum*. Metal chains were hung inside the pens to provide environmental enrichment throughout the trial. The trial house was pre-heated one day prior to the arrival of the piglets to achieve an ambient temperature of at least 21 degrees Celsius. The desired zone temperature of 28 degrees Celsius was achieved with the infrared lamps that were placed above the rubber mats as well as the installed diesel heater.

The trial started one week after arrival on the farm. During the one week adaption period, the piglets received the same creep feed that they have received on the farm before weaning. The feeding program was divided into creep, weaner, grower 1, grower 2 and finisher phases. Phase 1, the creep phase, commenced after the one-week adaptation period and consisted of a creep diet which was fed from the onset of the trial at 28 days of age until 49 days of age. The second phase consisted of a weaner diet that was fed from 49 days of age to 77 days of age. Phases 3 and 4 consisted of two grower diets, namely grower 1 and grower 2. Grower 1 was fed from 77 days of age to 105 days of age and Grower 2 was fed from 105 days of age until 133 days of age. Phase 5 consisted of a finisher diet containing ractopamine and was fed from 133 days of age to 154 days or slaughter. Energy, protein and lysine levels of each feeding phase were formulated to meet or exceed the requirements as set out by the NRC (2012) as well as the minimum specifications as set out by Act 36 of 1947 of the South African legislation.

3.4 Health management

The trial unit was cleaned and clear of pigs for approximately 6 months before the onset of the trial. Three weeks before the arrival of the piglets, the trial facility and flush channels were thoroughly cleaned with an antiseptic solution (Vircon, Lanxess, Germany). The process was repeated one week before the piglets arrived. The trial house was also disinfected with a veterinary disinfectant (F10SC, F10 products, South Africa) five days prior to the arrival of the piglets.

For the duration of the trial, strict hygiene measures were implemented to maintain a high level of biosecurity. Access was restricted at all times and access permission was only granted by the principal investigator. Overalls and gumboots were provided to workers and visitors and remained inside the pig house for the duration of the trial. Foot dip baths were provided outside the trial house and at the entrance in the trial facility once gumboots and overalls were put on. Overalls were washed frequently.

Pens contained partially slatted floors with underlying flush channels to remove manure collected under the slats. Manure that was not collected in the manure channels were scraped to the slats daily. A deep litter system was implemented throughout the trial. Drains were flushed as the levels in the channels increased to higher levels.

During the creep phase a number of piglets obtained PWD and severe cases were treated with a veterinary prescribed antibiotic solution (Peni LA, Virbac, South Africa). In these cases one mL of the antibiotic solution was injected intramuscularly once per piglet affected. Animals treated was monitored closely for recovery. During the growing period, pigs would sort dominance within a pen and a small number of pigs obtained leg injuries. These injuries healed quickly without the need for any treatment. A total of three mortalities were recorded for the whole trial period.

All medications were prescribed by the consulting veterinarian and deemed safe to inject. Piglets received a single and final intramuscular mycoplasma vaccination at 26 days of age as per commercial vaccination program and while still in the adaptation phase. In the event where animals had to be injected, it was done so without complications and in a safe time prior to slaughter.

3.5 Chemical analysis of feed samples

A representative feed sample was obtained from each of the trial diets (four treatments over five phases; 20 samples in total). These samples were analysed according to the proximate analysis system for their nutritional content as well as selected minerals. Samples were analysed at an accredited laboratory (Chemnutri Analytical Services, South Africa) and the nutrients analysed included dry matter (AOAC, 2000, Official method of analysis 942.05), crude protein (AOAC, 2000, Official method of analysis 988.05), lipids (AOAC, 2000, Official method of analysis 920.39), crude fibre (AOAC, 2000, Official method of analysis 962.09), ash (AOAC, 2000, Official method of analysis 942.05), total calcium (AOAC, 2000, Official method of analysis 935.13), total phosphorous (AOAC, 2000, Official method of analysis 965.17), magnesium (AOAC, 2000, Official method of analysis 984.27), potassium (AOAC, 2000, Official method of analysis 984.27) and sodium (AOAC, 2000, Official method of analysis 984.27).

3.6 Sampling and data collection

Production parameters were measured from the onset of the trial on a weekly basis and carcass parameters were measured at slaughter. Weekly data within a feed phase were combined and production data was reported per phase.

3.6.1 Production parameters

All parameters in the trail were measured on a fixed weekly bases and summarised per phase period at the transition from one phase to the following.

3.6.1.1 Feed intake

Pigs had *ad libitum* access to feed. Feed levels in the self-feeders were constantly monitored and replenished to ensure that the pigs were never without feed. At a fixed time weekly, feed from each pen would be weighed and subtracted from the amount of feed offered at the start of that particular production week. Feed levels of pens were individually monitored, separately weighed and recorded, before being placed into the feeder.

Feed intake was determined on a weekly basis (i.e. feed weighed in minus feed left after one week) and per feed phase. Left over feed was determined by weighing the whole feeder bin and subtracting the empty weight (tare weight) of the specific feeder bin. The left-over feed at the time of weighing was documented as the starting amount of feed for the new production week; except with transition to next feeding phase when left over feed was discarded. Negligible wastage of feed was noted during the trial. In the event of feed wastage, the wasted feed was immediately collected, weighed, recorded and subtracted from the amount of feed offered for the particular production week. One treatment at a time, starting with treatments without probiotics, was weighed, recorded and added to the feeders in an attempt to limit probiotic contamination between treatments. All feeders were closed during weighing to further limit dust contamination. At the end of a feeding phase period all the pen data generated was summarised and averaged for the period. This routine continued for the whole trial period and ended on the last production week the day before slaughter. Weekly feed intake, phase feed intake and cumulative feed intake over the total experimental period were calculated.

3.6.1.2 Body weight, body weight gain and average daily gain

All pigs were weighed using a platform scale on the same day as the weighing of the feeder bins. One pen at a time was weighed, with the three pigs in the particular pen being weighed individually and recorded. Weekly weight gain was calculated as well as average daily gain per pig for that particular week. Average daily gain (ADG) was calculated by dividing the total body weight gained per week or phase by the number of days in the production week or feeding phase. A pen average was calculated for both body weight (BW) and ADG by dividing the total value by the number of pigs in the pen.

3.6.1.3 Feed conversion ratio

Feed conversion ratio (FCR) was calculated by dividing the total feed consumed per pen per week and/or feeding phase by the total body weight gain for the particular period. Weekly FCR, phase FCR and cumulative FCR were calculated.

3.6.1.4 Faecal scoring

The faeces within a pen were scored on a weekly basis as an indication of the gut health of the pigs. All pens were scored based on the consistency and texture of fresh faeces within a pen. The following scoring criteria was used: 1 = normal hard faeces; 2 = slightly soft faeces; 3 = soft, partially formed faeces; 4 = loose, semi-liquid faeces; 5 = watery, mucous like faeces. Scoring was done by the same trained technician who was unaware of the dietary treatments applied in the trial.

3.6.2 Carcass parameters

Slaughtering took place at a fully accredited slaughter facility, exactly one day after the last data collection day of the final production week (Enterprise, Olifantsfontein, Gauteng, South Africa). All pigs were loaded and separated per treatment on slaughter day. Pigs were offloaded per treatment in pre-slaughter holding pens and allowed to relax after the transport from the trial facility. All treatments were slaughtered within one hour. Pigs were rendered unconscious by CO_2 asphyxiation and shortly thereafter hooked to the slaughter line and finally killed by exsanguination. All measurements and carcass parameters were conducted by the trained staff from Enterprise using accredited methods. Pigs were grouped together at the abattoir in treatment lots. Each slaughter lot contained all the animals within a particular treatment (mixed replicates within a treatment). Therefore carcass, and not pen, data was the experimental unit. Unfortunately, statistical analysis on dressing percentage was not possible due to the slaughter method at the abattoir. All test animals were slaughtered per treatment, but it was not possible to trace carcass weight back to the live weight of that specific animal. Due to the high slaughter rate within the abattoir it was very difficult to record pig identification numbers on the slaughter line.

3.6.2.1 Carcass classification

Carcasses were graded according to the official South Africa pork grading system (PORCUS), which is the industry standard grading system used. The grade under which the carcass is classified influences the price paid in ZAR/ kg for the carcass. The classification system is outlined in table 3.3.

Table 3.3. The South Africa pork carcass classification system

Classification of pork carcasses	Estimated % lean meat in carcass
Class P	70 and more
Class O	68-69
Class R	66-67
Class C	64-65
Class U	62-63
Class S	61 and less

3.6.2.2 Backfat thickness and fat content of carcasses at slaughter

Backfat thickness was measured as an indicator of lean meat percentage in live pigs using the Hennesey grading probe (Hennesey Grading Systems Ltd, New Zealand). The Hennesey probe is an opto-electric meat grading probe that is based on reflectance spectrometry. This probe is inserted at the P2 position, 60 mm from the backbone between the 3rd and 4th ribs, counting from the last rib. The measurement is taken while the carcass is hanging vertically and the duration of the measurement is one second. In this time, the probe records ten measurements a millimetre or up to 2,000 per second. Results were pooled per treatment and summarised.

3.6.2.3 Lean meat percentage of carcasses at slaughter

The percentage of lean meat in each carcass was measured immediately after slaughter using the Hennesey probe and a standardised measuring method as described in Section 3.6.2.2. Measurements were taken while the carcass was hanging vertically.

3.6.2.4 Carcass mass

The carcass of each animal was weighed with a hanging scale directly after slaughter with the head intact, but the GIT and surrounding organs removed to obtain the warm carcass mass. Cold carcass weight was also recorded 24 hours after slaughter. Carcasses were kept in a chill room at 6°C after slaughter.

3.7 Statistical analysis

A balanced experimental design was used in this trial assigning 14 pens to each treatment. Data was analysed statistically as a completely randomised block design with the GLM model (Statistical Analysis System, SAS, 2017) for the average effect over the time period of the trial. The randomised block design consisted of 14 blocks, each block consisted of one of each of the four different treatments. Treatments within blocks were assigned to two treatments (standard energy and reduced energy) and two subsequent levels (with probiotic and without probiotic). Repeated Measures Analysis of variance with the GLM model was used for repeated period measures. Means and standard errors were calculated and significance of difference ($P<0.05$) between means using the LS means test.

The linear model used is described by the following equation:

$$Y_{ij} = \mu + T_i + B_j + E_{ij}$$

$Y_{ij} = variable\ studied\ during\ the\ period$

$\mu = overall\ mean\ of\ the\ population$

$T_i = effect\ of\ the\ i^{th}\ treatment$

$B_j = effect\ of\ the\ j^{th}\ block$

$E_{ij} = error\ associated\ with\ each\ Y$

Statistical analysis for body weights and gains were calculated using starting body weight as a covariance, in order to account for variance in starting body weight that there might have been. The starting body weight between treatments did not differ at the start of the trial. Statistical analysis was completed for each production parameter measured. Each production parameter had two sets of data on which statistical analysis was completed. The first set of data contained all weekly data with measurements taken at the end of each production week and the second set contained the phase data which was compiled from the weekly data collected. Results were interpreted separately for the phase and weekly data.

LSMEANS was used to analyse the data, with $P \leq 0.05$ indicating significant difference and a tendency to be significant being recorded between $P > 0.05$ and $P \leq 0.10$.

3.8 Ethics approval

This project was approved for commencement by the Animal Ethics Committee of the University of Pretoria under the project approval number, EC051-17.

CHAPTER 4

RESULTS

4.1 Environmental conditions

The trial animals arrived early spring. Minimum ambient temperatures during this time were still relatively low compared to the rest of the trial period. During the duration of the trial, South Africa experienced two cold fronts and one heat wave. The first cold front was minor and happened during 12 and 13 weeks of age. The second cold front was more severe and lasted from 14 weeks of age until the pigs reached 15 weeks of age. One moderate heat wave occurred between the ages of 18 weeks until 19 weeks. The minimum and maximum house temperatures experienced during the trial are provided in Appendix 1.

4.2 Experimental diets

4.2.1 Colony Forming Unit's (CFU) in feed

Results of the CFU counts in probiotic containing feed after blending, are shown in table 4.1. A variation of 30% was regarded as acceptable according to Chr. Hansen's standards.

Table 4.1. Probiotic spore recovery in feed samples

Sample ID	Mean CFU/g	Expected CFU/g	Recovery (%)
Creep Treatment 1	1.4E+06	1.3E+06	109
Weaner Treatment 1	1.3E+06	1.3E+06	100
Grower 1 Treatment 1	9.1E+05	1.3E+06	70
Grower 2 Treatment 1	1.2E+06	1.3E+06	89
Finisher Treatment 1	1.4E+06	1.3E+06	110
Creep Treatment 3	1.2E+06	1.3E+06	94
Weaner Treatment 3	1.4E+06	1.3E+06	104
Grower 1 Treatment 3	1.4E+06	1.3E+06	108
Grower 2 Treatment 3	1.6E+06	1.3E+06	121
Finisher Treatment 3	1.1E+06	1.3E+06	84

CFU: Colony Forming Units

4.2.2 Chemical analyses of the trial feed

Samples of the trial feeds were analysed for dry matter, crude protein, fat, fibre, calcium, phosphorous, magnesium, potassium, sodium and ash content. The results of the chemical analyses of all feed samples are shown in table 4.2.

Table 4.2. Analysed nutrient composition (%) of experimental diets (as is basis)

	Creep				Weaner				Grower 1				Grower 2				Finisher			
	SE[1]		RE[2]		SE[1]		RE[2]		SE[1]		RE[2]		SE[1]		RE[2]		SE[1]		RE[2]	
Analysed Nutrients (%)	**+Pro[3]**	**-Pro[4]**	**+Pro[3]**	**-Pro[4]**	**+Pro[3]**	**-Pro[4]**	**+Pro[3]**	**-Pro[4]**	**+Pro[3]**	**-Pro[4]**	**+Pro[3]**	**-Pro[4]**	**+Pro[3]**	**-Pro[4]**	**+Pro[3]**	**-Pro[4]**	**+Pro[3]**	**-Pro[4]**	**+Pro[3]**	**-Pro[4]**
Dry matter	88.7	89.2	88.7	89.5	90.1	90.1	90.1	89.3	88.5	85.2	88.2	88.4	87.4	87.1	87.5	87.5	89.8	89.2	88.7	88.7
Moisture	11.3	10.8	11.3	10.4	9.9	9.9	9.9	10.7	11.5	14.8	11.8	11.6	12.6	12.9	12.5	12.5	10.2	10.8	11.3	11.3
Crude Protein	22.6	23.4	22.9	21.6	22.5	22.1	22.2	23.0	20.2	18.0	21.1	20.5	18.4	17.9	19.2	18.2	17.8	18.2	18.0	18.9
Fat	4.32	4.32	3.17	3.40	4.70	5.01	4.81	4.99	3.45	2.84	3.03	3.13	3.25	3.29	3.26	3.28	3.74	3.61	3.64	3.57
Fibre	2.41	2.51	2.54	2.56	2.52	2.54	2.64	2.89	2.42	2.78	2.82	2.79	3.40	3.29	3.67	3.60	3.16	4.15	3.46	3.34
Calcium	0.54	0.53	0.54	0.51	0.57	0.57	0.56	0.56	0.58	0.70	0.56	0.57	0.59	0.55	0.63	0.55	0.62	0.58	0.59	0.61
Phosphorus	0.38	0.38	0.39	0.40	0.46	0.46	0.49	0.49	0.43	0.43	0.46	0.48	0.40	0.39	0.42	0.40	0.44	0.41	0.41	0.45
Magnesium	0.14	0.15	0.15	0.15	0.14	0.14	0.16	0.16	0.16	0.16	0.18	0.18	0.18	0.17	0.19	0.19	0.18	0.18	0.19	0.20
Potassium	0.87	0.90	0.83	0.84	0.70	0.67	0.72	0.70	0.76	0.67	0.81	0.79	0.71	0.70	0.76	0.77	0.74	0.70	0.78	0.79
Sodium	0.24	0.24	0.26	0.26	0.20	0.20	0.20	0.21	0.24	0.25	0.24	0.25	0.25	0.24	0.25	0.23	0.24	0.23	0.23	0.24
Ash	5.31	5.22	5.27	5.28	4.76	4.81	4.82	4.89	4.48	4.69	4.53	4.75	4.43	4.39	4.79	4.59	4.56	4.43	4.72	4.81

[1] Standard energy (SE) diet

[2] Reduced energy (RE) diets contained 0.3 MJ/kg (72 kcal/kg) less net energy than the standard energy diet

[3] Added Bioplus YC probiotic at 400 g/ton of final feed

[4] Probiotic not added to diet

4.3 Production parameters

4.3.1 Body weight

4.3.1.1 Weekly body weight

Pigs were weighed weekly on the same day until the end of the trial. The body weight results of each treatment on a week-to-week basis can be viewed in table 4.3. No difference in body weight at the onset of the trial, after the adaptation period, was noted.

Dietary energy concentration had no significant effect on the body weight of pigs, measured weekly throughout the trial. Animals supplemented with the probiotic tended ($P<0.1$) to have significantly higher body weights at the first and second week of the trial when compared to non-supplemented animals. Pigs that received the probiotic in their feed were significantly heavier from week 6 onwards until slaughter. No interaction was recorded between energy level and probiotic inclusion in terms of body weight.

No difference in body weight was recorded between the standard and reduced energy diets treatments, without probiotics, for each week till slaughter (Treatments 2 and 4). When comparing the standard dietary energy level with and without the inclusion of a probiotic (Treatments 1 and 2), significant differences in body weight were noted during the first two weeks of the trial, thereafter the body weights did not differ till week 7. From week 8 onwards, significant differences in weekly body weights persisted till slaughter, which was within the first week of receiving the grower 1 diet. A tendency towards significant differences in body weight ($P<0.1$) for weekly results of Treatment 1 and 2, was observed at week 3 ($P=0.086$), week 6 ($P=0.095$) and week 7 ($P=0.064$).

The trend in differences in body weight between Treatments 1 and 2, was not the same for Treatments 3 and 4 (reduced energy diet with and without supplemented probiotics). Pigs that received the reduced energy diet had a slower body weight gain than that of the standard energy diets. Significant effects on body weight were recorded at week 10, 11, 12, 15 and 17. The trend ($P<0.1$) in body weight differences within the reduced energy group were only seen later when compared to standard energy group. A tendency towards significantly improved body weight was observed for Treatment 3 at week 8 ($P=0.077$), 14 ($P=0.053$) and 16 ($P=0.066$) when compared to Treatment 4. No differences in body weight were recorded between the high and low energy diets with added probiotic (Treatment 1 vs. Treatment 3).

Body weights of animals receiving the standard energy diet (Treatment 2) compared to the reduced energy with added probiotic (Treatment 3) indicated significant body weight differences at the end of week 1, 8, 9 and 10. During the last three weeks before slaughter, a strong tendency towards significantly heavier body weights ($P=0.052$-0.058) was recorded.

Table 4.3. The effect of probiotic supplementation on weekly body weight (kg) of pigs receiving diets with standard or reduced energy level

	Week of trial																	
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Dietary phase																	
Treatment	*Creep*				*Weaner*				*Grower 1*				*Grower 2*				*Finisher*	
SE + Probiotic (T1)[1]	7.14	9.33[a]	13.10[a]	16.88	21.67	26.78	33.14	39.38	47.22[a]	54.47[a]	60.90[a]	68.96[a]	77.39[a]	84.00[a]	91.67[a]	100.12[a]	111.06[a]	120.81[a]
SE (T2)[2]	7.11	9.06[b]	12.46[c]	16.39	21.09	26.17	32.17	38.08	44.95[c]	51.59[c]	58.43[b]	66.49[bc]	74.47[bc]	80.65[b]	88.40[b]	96.07[bc]	106.25[c]	115.74[bc]
RE + Probiotic (T3)[3]	7.12	9.35[a]	12.71[abc]	16.73	21.53	26.87	33.22	39.18	46.77[ab]	53.96[ab]	60.71[a]	68.36[ab]	76.65[ab]	82.57[ab]	90.59[ab]	99.22[ab]	109.95[abc]	119.62[ab]
RE (T4)[4]	7.09	9.27[ab]	12.56[bc]	16.69	21.13	26.29	32.14	38.18	45.28[bc]	52.54[abc]	58.45[b]	66.01[c]	74.01[c]	80.10[bc]	87.61[b]	95.59[c]	106.42[c]	115.60[c]
SEM[5]	0.192	0.090	0.185	0.195	0.271	0.316	0.402	0.481	0.580	0.727	0.741	0.757	0.803	0.972	1.056	1.136	1.318	1.371
Dietary effects (P value)																		
Energy		0.208	0.420	0.719	0.846	0.735	0.951	0.915	0.916	0.761	0.909	0.480	0.463	0.314	0.383	0.549	0.725	0.632
Probiotic		0.058	0.040	0.191	0.079	0.069	0.015	0.022	0.003	0.005	0.003	0.003	0.001	0.005	0.005	0.002	0.003	0.002
Energy x Probiotic		0.339	0.192	0.254	0.735	0.958	0.891	0.757	0.504	0.322	0.895	0.930	0.865	0.649	0.891	0.857	0.631	0.703

[a-c] Column means without common superscripts differ significantly (P≤0.05)

[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1

[2] Standard energy diet (no added probiotic), Treatment 2

[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3

[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4

[5]Standard Error of Means

4.3.1.2 Body weight at the end of each feeding phase

The results for body weight at the end of each feeding phase are shown in table 4.4.

Table 4.4. The effect of probiotic supplementation on phase body weight (kg) of pigs receiving diets with standard or reduced energy level

	Weeks of trial				
	0-4	4-8	8-12	12-16	16-18
	Dietary phase				
Treatment	*Creep*	*Weaner*	*Grower 1*	*Grower 2*	*Finisher*
SE + Probiotic (T1)[1]	16.88	39.38	68.97[a]	100.12[a]	120.82[a]
SE (T2)[2]	16.64	38.52	66.94[bd]	96.49[bc]	116.28[bc]
RE + Probiotic (T3)[3]	16.73	39.18	68.36[ad]	99.22[ab]	119.63[ac]
RE (T4)[4]	16.69	38.18	66.01[bc]	95.59[c]	115.60[b]
SEM[5]	0.190	0.433	0.707	1.098	1.366
Dietary effects (P value)					
Energy	0.769	0.533	0.283	0.417	0.496
Probiotic	0.472	0.038	0.004	0.002	0.003
Energy x Probiotic	0.598	0.876	0.822	0.996	0.854

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5] Standard Error of Means

Energy did not have a significant effect on the body weight of animals throughout the feeding phases (P=0.283-0.769). The effect of the inclusion of a probiotic in the feed was significant on body weight from the weaner phase onwards (P=0.002-0.038). This indicates that animals supplemented with a probiotic had significantly higher body weights from the weaner phase onwards till slaughter. No effect was recorded for the interaction between the energy level and probiotic inclusion.

Comparison between the non-supplemented standard energy diet and non-supplemented reduced energy diet (Treatment 2 and 4) indicated that there was no difference in body weight between the two energy levels. Although not significant, the standard energy diet indicated a marginally higher body weight than that of the reduced energy diet throughout the growing period.

In both energy levels (standard and reduced energy) the inclusion of a probiotic supplement significantly increased body weight of test animals from the grower phase onwards when compared to non-supplemented

animals. From the start of the trial, no difference in body weight was noted between standard and reduced energy diets supplemented with a probiotic additive, with the standard energy diet indicating marginally higher body weight at slaughter.

When comparing the standard energy diet with the reduced energy diet with supplemented probiotic (Treatment 2 and 3), body weights did not differ significantly, but results indicate a tendency towards higher body weight for animals receiving the reduced energy diet with added probiotic in the grower 2 and finisher phase (P=0.087 and P=0.092, respectively).

4.3.2 Average daily gain (ADG)

4.3.2.1 Weekly average daily gain performance

Weekly ADG performance results can be viewed in table 4.5.

Overall dietary energy concentration had no significant effect on weekly ADG performance measured throughout the trial, except for week 2. The effect of the probiotic on ADG of all animals supplemented vs. non-supplemented was not constant. Significantly higher ADG values from the probiotic supplemented animals were visible after week 6, 8, 15 and 17. No significant interaction between energy and probiotic inclusion was noted for weekly ADG.

On a week-to-week basis within treatments, no difference in ADG was recorded between the standard energy and reduced energy diets without added probiotic (Treatment 2 and 4). A tendency towards significant differences in ADG ($P<0.1$) for the weekly results of Treatment 2 and 4, was observed at week 10 (P=0.062).

Results between the standard energy and standard energy with an added probiotic (Treatment 1 and 2), only indicated a significant difference in ADG on week 8 and 17. The ADG of Treatment 1 was slightly higher than that of Treatment 2 for most of the trial. When comparing the reduced energy diet with the reduced energy with an added probiotic supplement (Treatment 3 and 4), Treatment 3 indicated a slightly higher ADG for most of the trial, although only week 6 was recorded as a significant difference in ADG between these two treatments. A tendency towards significant differences in ADG ($P<0.1$) for the weekly results of Treatment 3 and 4, was observed at week 4 (P=0.065) and week 10 (P=0.071).

Comparison between the results of the two density diets with added probiotics (Treatment 1 and 3), indicated no significant differences in ADG on a week-to-week basis.

Results between the standard energy diet and the reduced energy diet with an added probiotic supplement (Treatment 2 and 3), indicates a marginally better ADG for Treatment 3 on a weekly basis. Although there

were no constant significant differences, week 15 indicated significant improvement on ADG in Treatment 3 when compared to Treatment 2. A tendency towards significant differences in ADG ($P<0.1$) for the weekly results of Treatment 2 and 3, was observed at week 17 ($P=0.057$).

Table 4.5. The effect of probiotic supplementation on weekly average daily gain (ADG) of pigs receiving diets with standard or reduced energy level (kg/pig/day)

	Week of trial																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Dietary phase																
Treatment	*Creep*			*Weaner*				*Grower 1*				*Grower 2*				*Finisher*	
SE + Probiotic (T1)[1]	0.316	0.540[a]	0.540	0.685	0.729	0.909[a]	0.891	1.120[a]	1.035	0.919	1.152	1.203	0.945	1.095	1.206[ab]	1.563	1.393[a]
SE (T2)[2]	0.299	0.498[ab]	0.564	0.674	0.731	0.865[ab]	0.855	0.980[c]	0.961	0.969	1.063	1.152	0.873	1.109	1.089[b]	1.489	1.264[b]
RE + Probiotic (T3)[3]	0.320	0.479[b]	0.574	0.686	0.763	0.908[a]	0.851	1.085[ab]	1.027	0.965	1.092	1.185	0.845	1.145	1.233[a]	1.533	1.382[ab]
RE (T4)[4]	0.307	0.470[b]	0.590	0.634	0.737	0.835[b]	0.863	1.014[bc]	1.037	0.843	1.082	1.142	0.871	1.073	1.140[ab]	1.547	1.312[ab]
SEM[5]	0.010	0.020	0.019	0.019	0.014	0.024	0.024	0.032	0.044	0.046	0.041	0.042	0.050	0.050	0.049	0.048	0.042
Dietary effects (P value)																	
Energy	0.523	0.030	0.119	0.329	0.164	0.510	0.510	0.982	0.446	0.394	0.611	0.743	0.312	0.870	0.436	0.780	0.669
Probiotic	0.124	0.211	0.286	0.113	0.401	0.019	0.614	0.002	0.478	0.447	0.236	0.269	0.644	0.522	0.039	0.540	0.024
Energy x Probiotic	0.810	0.412	0.828	0.295	0.330	0.552	0.316	0.282	0.352	0.071	0.345	0.924	0.327	0.341	0.802	0.365	0.491

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5]Standard Error of Means

4.3.2.2 Average daily gain performance at the end of each feeding phase

The results for ADG performance at the end of each feeding phase are presented in table 4.6.

Table 4.6. The effect of probiotic supplementation on dietary phase average daily gain (ADG) of pigs receiving diets with standard or reduced energy level (kg/pig/day)

	Weeks of trial				
	1-4	4-8	8-12	12-16	16-18
	Dietary phase				
Treatment	*Creep*	*Weaner*	*Grower 1*	*Grower 2*	*Finisher*
SE + Probiotic (T1)[1]	0.465	0.804[a]	1.056[a]	1.113	1.478[a]
SE (T2)[2]	0.454	0.781[ab]	0.993[c]	1.056	1.377[b]
RE + Probiotic (T3)[3]	0.458	0.802[a]	1.042[ac]	1.102	1.457[ab]
RE (T4)[4]	0.455	0.767[b]	0.994[bc]	1.056	1.430[ab]
SEM[5]	0.001	0.011	0.019	0.021	0.030
Dietary effects (P value)					
Energy	0.771	0.506	0.722	0.827	0.605
Probiotic	0.470	0.018	0.006	0.020	0.041
Energy x Probiotic	0.596	0.604	0.696	0.795	0.234

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5] Standard Error of Means

Energy did not have a significant effect on the feeding phase ADG of animals receiving standard and reduced energy diets. The dietary effect of the supplemented probiotic was significant in the weaner, grower 1, grower 2 and finisher phases in terms of ADG, when comparing all supplemented vs non-supplemented animals. The probiotic did not have an effect on ADG in the creep phase when comparing all supplemented vs non-supplemented animals. No significant effect on ADG was recorded with the probiotic and energy interaction.

No significant difference in ADG per phase was recorded between the standard and reduced energy diets without supplemented probiotic (Treatment 2 and 4).

ADG comparison within the standard energy diets, with and without supplemented probiotic (Treatment 1 and 2), indicated a significantly improved ADG in the grower 1 and finisher phases for the probiotic supplemented treatment (Treatment 1) over the non-supplemented group (Treatment 2). A tendency towards significant differences in ADG (P<0.1) for the feeding phase results of Treatment 1 and 2, was observed at the grower 2 phase (P=0.065). The same comparison in the reduced energy group, indicated a significant improvement in

the ADG for the weaner phase of the probiotic supplemented group (Treatment 3) over the non-supplemented group (Treatment 4). A tendency towards significant differences in ADG ($P<0.1$) for the phase results of Treatment 3 and 4, was observed at the grower 1 feeding phase ($P=0.084$).

Standard and reduced energy diets with supplemented probiotic (Treatment 1 and 3), indicated no significant difference in terms of ADG on a phase-to-phase basis.

A comparison between the standard energy diet and the reduced energy diet with supplemented probiotic (Treatment 2 and 3), indicated no significant difference in ADG throughout the growing period. A tendency towards significant differences in ADG ($P<0.1$) for the phase results of Treatment 2 and 3, was observed at the grower 1 ($P=0.08$) and finisher phases ($P=0.068$).

4.3.3 Average daily feed intake (ADFI)

4.3.3.1 Weekly average daily feed intake performance

The results for the weekly ADFI performance can be viewed in table 4.7.

The energy content of trial diets, on a week-to-week basis did not have a constant significant effect on the ADFI of animals. Energy level did have an effect on the feed intake during week 17, with animals receiving a reduced energy diet having a higher ADFI. If one observes the dietary effect of the probiotic on the ADFI of all animals supplemented vs. non-supplemented, results indicate only a significant effect at the onset of the grower 1 phase at week 8. Animals supplemented with a probiotic did have a higher feed intake at week 8 vs. non supplemented animals. Energy x probiotic interaction did not affect ADFI.

On a week-to-week basis within treatments, no significant difference in ADFI per week was recorded between the standard and reduced energy diets without supplemented probiotic (Treatment 2 and 4) except for final week (week 17), where the standard energy diet showed a significantly lower feed intake.

ADFI within the standard energy diets, with and without supplemented probiotic (Treatment 1 and 2), did not differ throughout the trial. The same comparison for the reduced energy diets, with and without supplemented probiotic (Treatment 3 and 4), also indicated no significant differences in ADFI throughout the trial.

Standard and reduced energy diets with supplemented probiotic (Treatment 1 and 3), did not differ in terms of ADFI on a week-to-week basis throughout the trial.

Significant differences were noted for ADFI after weeks 5, 7 and 17 between pigs that received a standard energy diet and the reduced energy diet with supplemented probiotic (Treatment 2 and 3). Reduced energy, probiotic supplemented animals had a significantly higher ADFI at weeks 5, 7 and 17 than animals of Treatment 2. A tendency towards significant differences in ADFI ($P<0.1$) for Treatment 2 and 3, was observed at weeks 6 ($P=0.07$) and 8 ($P=0.071$).

Table 4.7. The effect of probiotic supplementation on weekly average daily feed intake (ADFI) of pigs receiving diets with standard or reduced energy level (kg/pig/day)

	Week of trial																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Dietary phase																
Treatment	*Creep*			*Weaner*				*Grower 1*				*Grower 2*				*Finisher*	
SE + Probiotic (T1)[1]	0.345	0.635	0.757	0.877	1.053[ab]	1.283	1.491[ab]	1.694	1.921	1.861	2.299	2.350	2.244	2.612	2.764	2.945	3.008[ab]
SE (T2)[2]	0.338	0.597	0.729	0.872	1.019[b]	1.247	1.401[b]	1.600	1.837	1.819	2.193	2.344	2.194	2.663	2.749	2.845	2.927[b]
RE + Probiotic (T3)[3]	0.343	0.605	0.764	0.911	1.111[a]	1.340	1.515[a]	1.711	1.910	1.924	2.295	2.377	2.235	2.754	2.861	3.037	3.113[a]
RE (T4)[4]	0.330	0.586	0.745	0.894	1.068[ab]	1.258	1.483[ab]	1.633	1.845	1.848	2.270	2.332	2.252	2.703	2.775	2.961	3.110[a]
SEM[5]	0.009	0.022	0.026	0.029	0.030	0.035	0.035	0.042	0.049	0.053	0.063	0.054	0.069	0.067	0.066	0.081	0.057
Dietary effects (P value)																	
Energy	0.631	0.350	0.913	0.338	0.086	0.339	0.138	0.561	0.971	0.393	0.567	0.889	0.725	0.186	0.354	0.210	0.016
Probiotic	0.280	0.191	0.590	0.712	0.213	0.103	0.090	0.048	0.139	0.273	0.304	0.640	0.817	0.995	0.449	0.287	0.468
Energy x Probiotic	0.785	0.677	0.618	0.827	0.884	0.511	0.406	0.842	0.854	0.756	0.529	0.715	0.632	0.449	0.593	0.881	0.497

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5]Standard Error of Means

4.3.3.2 Average daily feed intake over each feeding phase

The ADFI results for each dietary phase are presented in table 4.8.

Table 4.8. The effect of probiotic supplementation on average daily feed intake (ADFI) over phases for pigs receiving diets with standard or reduced energy level (kg/pig/day)

	Weeks of trial				
	1-4	4-8	8-12	12-16	16-18
	Dietary phase				
Treatment	*Creep*	*Weaner*	*Grower 1*	*Grower 2*	*Finisher*
SE + Probiotic (T1)[1]	0.579	1.176[ab]	1.944	2.492	2.977[ab]
SE (T2)[2]	0.555	1.135[b]	1.863	2.488	2.886[b]
RE + Probiotic (T3)[3]	0.565	1.219[a]	1.960	2.557	3.075[a]
RE (T4)[4]	0.554	1.176[ab]	1.899	2.515	3.036[ab]
SEM[5]	0.014	0.029	0.044	0.050	0.059
Dietary effects (P value)					
Energy	0.606	0.152	0.553	0.357	0.043
Probiotic	0.219	0.152	0.113	0.644	0.279
Energy x Probiotic	0.648	0.967	0.816	0.712	0.665

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5] Standard Error of Means

The dietary effect of energy content of trial diets between dietary phases was not constant on the ADFI of animals on the standard vs. reduced energy diets. For the finisher phase, dietary energy level had a significant effect on the ADFI of animals. All animals on the reduced energy diets indicated a significantly higher ADFI in the finisher phase. Neither probiotic, nor the interaction between energy level and probiotic inclusion had any effect on phase ADFI.

Within treatments, no significant difference in ADFI was recorded between the standard and reduced energy diets without supplemented probiotic (Treatment 2 and 4) during different feed phases. A tendency towards significant differences in ADFI ($P<0.1$) for Treatment 2 and 4, was observed for the finisher phase ($P=0.081$).

Phase ADFI comparison within the standard energy diets, with and without supplemented probiotic (Treatment 1 and 2), indicated no significant differences in ADFI throughout the trial. The same comparison for the reduced energy diets, with and without supplemented probiotic (Treatment 3 and 4), also indicated no significant differences in ADFI throughout the trial.

Standard and reduced energy diets with supplemented probiotic (Treatment 1 and 3), indicated no difference in terms of ADFI on a phase-to-phase basis throughout the trial.

A comparison between the standard energy diet and the reduced energy diet with supplemented probiotic (Treatment 2 and 3), indicated significant differences in ADFI at the weaner and finisher phases. Reduced energy, probiotic supplemented animals had a significantly higher ADFI at weaner and finisher phases than animals of Treatment 2.

4.3.3.3 Cumulative feed intake per phase

The cumulative feed intake results per phase are presented in table 4.9.

Table 4.9. The effect of probiotic supplementation on cumulative feed intake of pigs receiving diets with standard or reduced energy level (kg/pig)

	Weeks of trial				
	1-4	1-8	1-12	1-16	1-18
	Dietary phase				
Treatment	*Creep*	*Weaner*	*Grower 1*	*Grower 2*	*Finisher*
SE + Probiotic (T1)[1]	12.158	45.085	99.513	169.297	210.971
SE (T2)[2]	11.647	43.421	95.572	165.226	205.629
RE + Probiotic (T3)[3]	11.865	46.011	100.885	172.478	215.527
RE (T4)[4]	11.629	44.555	97.726	168.157	210.658
SEM[5]	0.299	1.048	2.178	3.153	3.732
Dietary effects (P value)					
Energy	0.606	0.332	0.423	0.336	0.207
Probiotic	0.219	0.145	0.111	0.191	0.180
Energy x Probiotic	0.648	0.922	0.859	0.869	0.950

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5] Standard Error of Means

The dietary effect of energy content of trial diets between dietary phases was not constant on the cumulative feed intake of animals. The probiotic and the interaction between energy and probiotic inclusion, had no effect on the cumulative feed intake over the various phases of trial animals.

No significant difference in cumulative feed intake per phase was recorded between the standard and reduced energy diets without supplemented probiotic (Treatment 2 and 4).

Cumulative feed intake over phases within the standard energy diets, with and without supplemented probiotic (Treatment 1 and 2), indicated no significant differences throughout the trial, similar to the findings of the reduced energy diets, with and without supplemented probiotic (Treatment 3 and 4).

Standard and reduced energy diets with supplemented probiotic (Treatment 1 and 3), indicated no significant difference in terms of cumulative feed intake, but a tendency towards significantly higher cumulative feed intake was observed in the weaner, grower and finisher phases for Treatment 3 (P=0.089, P=0.092, P=0.068 respectively).

4.3.4 Feed conversion ratio (FCR)

4.3.4.1 Weekly feed conversion ratio performance

Weekly FCR performance results can be viewed in table 4.10.

The energy content of trial diets did not have a constant effect on FCR of animals. Energy level did have an effect on the FCR during week 4 and 6 with animals receiving a standard energy diet having a better FCR. If one observes the dietary effect of the probiotic on the FCR of all animals supplemented vs. non-supplemented, results indicate only a significant effect at the onset of the grower 1 phase at week 8. Animals supplemented with a probiotic did have a better FCR at week 8 vs. non supplemented animals. No energy and probiotic interaction effects on weekly FCR were noted.

No significant difference in FCR per week was recorded between the standard and reduced energy diets without supplemented probiotic (Treatment 2 and 4) except for the first week of the weaner phase (week 4), where a standard energy diet resulted in a significantly better FCR. A tendency towards significant differences in FCR ($P<0.1$) for Treatment 2 and 4, was observed at weeks 6 (P=0.081) and 10 (P=0.068).

FCR of pigs that received feeds with standard energy with probiotic supplementation had a significantly lower FCR at the end of week 8 than those without a probiotic. A tendency towards significant differences in FCR ($P<0.1$) for Treatment 1 and 2 was observed at week 15 (P=0.09) while for the reduced energy diets, with and without supplemented probiotic (Treatment 3 and 4), the only significant difference in FCR was noted at week 4 at the onset of the weaner phase.

Standard and reduced energy diets with supplemented probiotic (Treatment 1 and 3), only differed significantly in terms of FCR in week 13.

A comparison between the standard energy diet and the reduced energy diet with supplemented probiotic (Treatment 2 and 3) indicated no significant differences in terms of FCR performance on a weekly basis.

Table 4.10. The effect of probiotic supplementation on weekly feed conversion ratio (FCR) of pigs receiving diets with standard or reduced energy level

	Week of trial																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Dietary phase																
Treatment	*Creep*			*Weaner*				*Grower 1*				*Grower 2*				*Finisher*	
SE + Probiotic (T1)[1]	1.101	1.180	1.400[b]	1.289[a]	1.452	1.412[a]	1.693	1.526[a]	1.872	2.034	2.026	1.987	2.416[a]	2.430	2.327	1.889	2.197[a]
SE (T2)[2]	1.141	1.215	1.283[a]	1.293[a]	1.405	1.429[ab]	1.671	1.653[b]	1.907	1.940	2.098	2.075	2.497[ab]	2.354	2.597	1.919	2.344[ab]
RE + Probiotic (T3)[3]	1.076	1.272	1.312[ab]	1.331[a]	1.461	1.485[ab]	1.797	1.586[ab]	1.938	2.053	2.120	2.023	2.840[b]	2.499	2.355	1.991	2.286[ab]
RE (T4)[4]	1.104	1.291	1.305[ab]	1.419[b]	1.453	1.526[b]	1.734	1.618[ab]	1.816	2.971	2.133	2.055	2.643[ab]	2.532	2.491	1.943	2.401[b]
SEM[5]	0.036	0.058	0.041	0.030	0.027	0.038	0.050	0.037	0.091	0.388	0.078	0.065	0.143	0.113	0.110	0.056	0.068
Dietary effects (P value)																	
Energy	0.401	0.158	0.422	0.008	0.312	0.032	0.109	0.749	0.889	0.184	0.410	0.901	0.053	0.281	0.723	0.267	0.295
Probiotic	0.357	0.652	0.136	0.134	0.324	0.445	0.405	0.034	0.632	0.296	0.584	0.361	0.689	0.848	0.072	0.880	0.063
Energy x Probiotic	0.871	0.889	0.183	0.174	0.482	0.758	0.689	0.207	0.392	0.200	0.707	0.666	0.337	0.630	0.543	0.483	0.819

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5]Standard Error of Means

4.3.4.2 Feed conversion ratio results at the end of each feeding phase

The dietary phase FCR performance results can be viewed in table 4.11.

Table 4.11. The effect of probiotic supplementation on feed conversion ratio (FCR) of pigs receiving diets with standard or reduced energy level for each feeding phase

	Weeks of trial				
	1-4	4-8	8-12	12-16	16-18
	Dietary phase				
Treatment	*Creep*	*Weaner*	*Grower 1*	*Grower 2*	*Finisher*
SE + Probiotic (T1)[1]	1.240	1.462[a]	1.839[a]	2.244[a]	2.022[a]
SE (T2)[2]	1.217	1.452[a]	1.880[ab]	2.321[ab]	2.104[ab]
RE + Probiotic (T3)[3]	1.234	1.521[b]	1.882[ab]	2.322[ab]	2.115[b]
RE (T4)[4]	1.225	1.537[b]	1.918[b]	2.386[b]	2.133[bc]
SEM[5]	0.052	0.023	0.028	0.029	0.032
Dietary effects (P value)					
Energy	0.905	0.001	0.137	0.017	0.057
Probiotic	0.393	0.907	0.153	0.019	0.117
Energy x Probiotic	0.727	0.522	0.920	0.821	0.303

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5] Standard Error of Means

The energy content of trial diets had significant effects on the FCR of the weaner and grower 2 phases of animals on the standard vs. reduced energy diets, with a standard energy diet resulting in a better FCR. Between the probiotic's effect on FCR of animals supplemented vs. non-supplemented, only a significant effect on FCR was found at the grower 2 phase. No significant energy x probiotic interaction on FCR, was noted.

A significant difference in FCR was only recorded between the standard and reduced energy diets without supplemented probiotic (Treatment 2 and 4) at the weaner phase, where a standard energy diet indicated a significantly better FCR.

Within the standard energy diets, with and without supplemented probiotic (Treatment 1 and 2), no significant differences in FCR throughout the trial were indicted, similar for the reduced energy diets, with and without supplemented probiotic (Treatment 3 and 4).

Standard and reduced energy diets with supplemented probiotic (Treatment 1 and 3), indicated significant differences in terms of FCR in the weaner and finisher phases. A standard energy diet with the supplemented probiotic resulted in a better FCR in both the weaner and finisher phases when compared to a reduced energy diet with a supplemented probiotic. Also, a tendency towards a significant difference in FCR ($P<0.1$) between Treatment 1 and 3, was observed at grower 2 ($P=0.067$) phase.

A comparison between the standard energy diet and the reduced energy diet with supplemented probiotic (Treatment 2 and 3), only showed significant differences in FCR at the weaner phase.

4.3.4.3 Cumulative phase FCR results

The dietary phase FCR cumulative performance results can be viewed in table 4.12.

Table 4.12. The effect of probiotic supplementation on cumulative feed conversion ratio (FCR) of pigs receiving diets with standard or reduced energy level for each feeding phase

	Weeks of trial				
	1-4	1-8	1-12	1-16	1-18
	Dietary phase				
Treatment	*Creep*	*Weaner*	*Grower 1*	*Grower 2*	*Finisher*
SE + Probiotic (T1)[1]	1.240	1.395^{ab}	1.607^{a}	1.819^{a}	1.855^{a}
SE (T2)[2]	1.217	1.380^{a}	1.612^{a}	1.842^{ab}	1.886^{ab}
RE + Probiotic (T3)[3]	1.234	1.435^{b}	1.646^{ab}	1.872^{bc}	1.916^{bc}
RE (T4)[4]	1.225	1.438^{b}	1.664^{b}	1.904^{c}	1.945^{c}
SEM[5]	0.018	0.015	0.017	0.017	0.017
Dietary effects (P value)					
Energy	0.950	0.002	0.010	0.002	0.001
Probiotic	0.393	0.681	0.512	0.121	0.087
Energy x Probiotic	0.727	0.547	0.727	0.801	0.994

$^{a-c}$ Column means without common superscripts differ significantly ($P\leq0.05$)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5] Standard Error of Means

The energy content of trial diets had significant effects on the cumulative FCR of pigs for the weaner, grower 1, grower 2 and finisher phases, thus indicating that animals receiving a standard energy diet had a better cumulative FCR. FCR of animals which were supplemented vs. non-supplemented with probiotic, only tended towards a significant effect at the finisher phase. Animals supplemented with a probiotic did have a marginally

better cumulative FCR from the grower 1 phase vs. non supplemented animals. No interaction effect between energy x probiotic was found.

When comparing pigs that received different energy levels without probiotic supplementation, cumulative FCR was significantly better for the pigs that received the standard energy for the period from the weaner phase until slaughter (Treatment 2 and 4).

FCR comparison within the standard energy diets, with and without supplemented probiotic (Treatment 1 and 2), indicated no significant differences in cumulative FCR throughout the trial. The standard energy probiotic supplemented group (Treatment 1) had a marginally better cumulative FCR when compared to the standard energy non-supplemented group (Treatment 2) from the grower 1 phase onwards. The same comparison for the reduced energy diets, with and without supplemented probiotic (Treatment 3 and 4), also indicated no significant differences in cumulative FCR throughout the trial. The reduced energy, probiotic supplemented group (Treatment 3) resulted in a marginally better cumulative FCR when compared to the reduced energy non-supplemented group (Treatment 4) from the grower 1 phase onwards.

Pigs that received the standard and reduced energy diets with supplemented probiotic (Treatment 1 and 3), differed significantly in terms of cumulative FCR in the grower 2 and finisher phases. A standard energy diet with the supplemented probiotic resulted in a better cumulative FCR from the grower 2 phase onwards when compared to a reduced energy diet with a supplemented probiotic. Also, a tendency towards significant differences in cumulative FCR ($P<0.1$) for Treatment 1 and 3, was observed at the weaner phase ($P=0.067$).

A comparison between the standard energy diet and the reduced energy diet with supplemented probiotic (Treatment 2 and 3), only indicated significant differences in cumulative FCR at the weaner phase.

4.3.5 Carcass characteristics

Slaughter parameter results per treatment were obtained from the abattoir for each treatment group. The slaughter parameters per treatment are presented in table 4.13.

Table 4.13. The effect of probiotic supplementation on slaughter parameters of pigs receiving diets with standard or reduced energy level

Treatment	*Warm carcass weight (kg)*	*Back fat thickness (mm)*	*Muscle thickness (mm)*	*Lean meat percentage (%)*
SE + Probiotic (T1)[1]	91.10[a]	13.76	52.30	69.01
SE (T2)[2]	88.70[ac]	14.00	51.61	68.86
RE + Probiotic (T3)[3]	90.83[ab]	13.58	51.40	69.69
RE (T4)[4]	84.95[c]	13.35	50.57	69.11
SEM[5]	1.473	0.394	0.854	0.379
Dietary effects (P value)				
Energy	0.177	0.289	0.259	0.223
Probiotic	0.006	0.987	0.381	0.336
Energy x Probiotic	0.242	0.987	0.931	0.576

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5] Standard Error of Means

4.3.5.1 Warm carcass weight

The energy content of trial diets did not have a significant effect on the carcass weight of animals. However, significantly heavier carcasses for probiotic supplemented animals were achieved. There was no energy x probiotic interaction effect noted for carcass weight.

When observing within treatments, no significant difference in carcass weight was recorded between the standard and reduced energy diets without supplemented probiotic (Treatment 2 and 4). A tendency towards significant differences in carcass weight (P<0.1) for Treatment 2 and 4, was observed (P=0.077).

When comparing carcass weight results within the standard energy diets, with and without supplemented probiotic (Treatment 1 and 2), no significant differences in carcass weight were recorded, even though the probiotic supplemented group was able to produce a 2.4 kg heavier carcass on average. The same comparison for the reduced energy diets, with and without supplemented probiotic (Treatment 3 and 4), indicated a significantly heavier carcass when a reduced energy diet was supplemented with a probiotic additive and average gains resulted in a 5.88 kg heavier carcass.

Standard and reduced energy diets with supplemented probiotic (Treatment 1 and 3), did not differ in terms of carcass weight at slaughter.

There was no significant difference in carcass weight between the standard energy diet and the reduced energy diet with supplemented probiotic (Treatment 2 and 3). A reduced energy diet with an added probiotic resulted in 2.13 kg heavier carcasses on average at slaughter when compared to a standard energy diet without supplemented probiotic.

4.3.5.2 Back fat thickness, muscle thickness and lean meat percentage.

No significant differences in back fat thickness, muscle thickness and lean meat percentage of carcasses between any of the treatments, were noted.

4.3.6 Faecal score

The faecal score results are shown in table 4.14.

Table 4.14. The effect of probiotic supplementation on faecal score of pigs receiving diets with standard or reduced energy level per dietary phase

	Weeks of trial				
	1-4	4-8	8-12	12-16	16-18
	Dietary phase				
Treatment	*Creep*	*Weaner*	*Grower 1*	*Grower 2*	*Finisher*
SE + Probiotic (T1)[1]	1.686	1.446[a]	1.554[ab]	1.589	1.571
SE (T2)[2]	1.857	1.554[ab]	1.411[a]	1.661	1.429
RE + Probiotic (T3)[3]	1.692	1.614[ab]	1.715[b]	1.654	1.600
RE (T4)[4]	2.024	1.804[b]	1.643[ab]	1.750	1.607
SEM[5]	0.122	0.110	0.084	0.108	0.121
Dietary effects (P value)					
Energy	0.768	0.067	0.027	0.487	0.405
Probiotic	0.110	0.189	0.215	0.450	0.584
Energy x Probiotic	0.298	0.712	0.682	0.911	0.544

[a-c] Column means without common superscripts differ significantly (P≤0.05)
[1] Standard energy diet + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 1
[2] Standard energy diet (no added probiotic), Treatment 2
[3] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) + Bioplus YC at 400 g/ton (3.2E+09 /g CFU), Treatment 3
[4] Reduced energy diet (0.3 MJ/kg or 72 kcal/kg on NE level) no added probiotic, Treatment 4
[5] Standard Error of Means

Energy had a significant effect on the faecal score during the grower 1 phase, with pigs receiving a standard energy diet having a slightly better score than pigs receiving the reduced energy diets. Probiotic

supplementation had no significant effect on the faecal score. No interaction effect between energy and probiotic supplementation was noted either.

No significant differences in the faecal score were recorded between the standard and reduced energy diets without supplemented probiotic (Treatment 2 and 4). A tendency towards significant differences in the faecal score ($P<0.1$) for Treatment 2 and 4, was observed at the grower 1 phase ($P=0.068$).

Faecal scoring comparison within the standard energy diets, with and without supplemented probiotic (Treatment 1 and 2), showed no significant differences in faecal score throughout the trial. The same comparison for the reduced energy diets, with and without supplemented probiotic (Treatment 3 and 4), also indicated no significant differences in the faecal score throughout the trial. A tendency towards a significant difference in faecal score ($P<0.1$) for Treatment 3 and 4, was observed in the weaner phase ($P=0.059$).

No significant differences in terms of faecal score between the standard and reduced energy diets with supplemented probiotic (Treatment 1 and 3) were shown.

A comparison between the standard energy diet and the reduced energy diet with supplemented probiotic (Treatment 2 and 3), revealed a significant difference in faecal score at the grower 1 phase.

CHAPTER 5
DISCUSSION

The main potential benefits of supplementing monogastric animal diets with probiotic feed additives are well documented and supported by literature (Casula & Cutting, 2002; Oelschlaeger, 2010; Soccol *et al.*, 2010; Brown, 2011; Cutting, 2011). Supplementation of *Bacillus*-based probiotics in pigs has previously resulted in performance benefits which included improved feed efficiency, mortality and weight gain in weaned piglets (De Lange *et al.*, 2010; Le Bon *et al.*, 2010; Patil *et al.*, 2015; Jørgensen *et al.*, 2016). Improved digestibility, growth performance and feed conversion in growing pigs are documented (Alexopoulos *et al.*, 2004; Londoño *et al.*, 2016), as well as improvements in feed efficiency, weight gain and carcass quality of finisher pigs (Alexopoulos *et al.*, 2004; FAO, 2016; Jørgensen *et al.*, 2016).

5.1 Standard energy vs reduced energy diets

In order to evaluate whether a probiotic can contribute energy towards the pig diet during a growth performance trial, pigs should be exposed to an energy deficiency in order to cause a reduction in performance. For most of the parameters measured, the pigs that received the standard energy diets did not perform significantly different from those that received the reduced energy diets (Treatment 2 and 4). The reduced energy diet (Treatment 4) was still able to support a high growth rate close to the genetic potential of the trial pigs. Therefore, the hypothesis that the probiotic can improve energy digestibility could not be tested in the current study. The trial diets were formulated at high energy levels commonly used in commercial piggeries. Although a marginal reduction in production performance was noted for pigs on the reduced energy diets, the difference in energy between the diets was not sufficient and should have been more than the 0.3 MJ/kg or 72 kcal/kg on NE level that was used in this trial. It is likely that pigs inside the trial facility experienced more favourable conditions than that typically encountered in commercial piggeries, even though stocking density within the trial pens were moderate. It is also probable that the pigs receiving the reduced energy diets could have spent enough time at the feeders to compensate for the marginal lower dietary energy. Even if the probiotic was able to increase the energy availability of the feed, the animals could not have used the additional energy for growth as they were already growing close to the genetic potential. A retrospective analysis study conducted by Young *et al.* (2003) compared the difference of two energy density diets (2.5% and 5% added fat) in university and field research facilities. The results showed that field research facilities had a 30% lower ADFI and a better response in FCR towards supplemented energy than that of university research facilities, thus indicating near optimal growth in the university facilities.

5.2 Body weight and average daily gain

The probiotic had a significant influence on the body weight of pigs. Body weight gain of probiotic supplemented pigs was higher than the unsupplemented pigs as early as the creep phase and continued to improve at a constant rate in the weaner phase. Probiotic supplementation had a significant positive effect on the body weight of pigs within both the dietary energy groups at the onset of the grower 1 phase. These findings are corresponding with the findings of Alexopoulos *et al.* (2004), Cho *et al.* (2011), Zimmermann *et al.* (2016) and Lan *et al.* (2017), who also found that probiotic supplementation in feed improved the growth rate in growing pigs.

The inclusion of a probiotic in the standard energy diet (Treatment 1 vs. 2) significantly increased the body weight of the pigs from week 8 onwards, until slaughter. Pigs that received the reduced energy diet with the added probiotic were also heavier than those without the probiotic supplementation (Treatment 3 vs. 4) from week 10 onwards until week 12. The effect of the probiotic on body weight was not constant for the different energy densities and the probiotic had a more pronounced effect on body weight of the pigs that received the standard energy diet. This observation is in agreement with the statement of Meng *et al.* (2010) that a higher nutrient density could have optimised the gut microbiota balance, resulting in improved utilisation of nutrients. It is possible that the supplementation of a probiotic could affect the absorption of nutrients in the pig gut. Cai *et al.* (2015) investigated the effect of a probiotic on gut health in nursery pigs and found that pigs fed a probiotic had longer duodenum, jejunum and ileal villi when compared to control pigs. The cumulative positive effect on the gut villi thickness and surface area with the addition of a probiotic could support increased absorption of digested nutrients and subsequently increase animal performance (Al-Baadani *et al.*, 2016).

The addition of a probiotic to both the standard energy and reduced energy diets significantly improved the ADG from the weaner phase onwards until slaughter, when compared to the non-supplemented animals. ADG performance during the creep phase was not significantly affected by the addition of a probiotic. This corresponds with the findings of Jaworski *et al.* (2017) and Jørgensen *et al.* (2016) who also found no effect of a probiotic on ADG in the post weaning phase. The standard energy diets with added probiotic (Treatment 1) resulted in significantly improved ADG when compared to the standard energy diet without the probiotic (Treatment 2) in the grower and finisher phases. A tendency towards better ADG in the grower 2 phase was also noted. However, the reduced energy diet with (Treatment 3) and without (Treatment 4) supplemented probiotic did not yield the same results. Other than the differences found during the weaner phase and a tendency in the grower 1 phase, no significant differences in ADG was recorded between pigs that received the reduced energy diet with supplemented probiotic (Treatment 3) and reduced energy diet without probiotic (Treatment 4). The probiotic supplemented group did however have a numerically higher ADG. As previously mentioned, it could be possible that the diet composition affects the outcome of a probiotic in growing pigs

(Meng *et al.*, 2010). Increased growth rates can be attributed to several direct and indirect effects as a result of probiotic supplementation. *Bacillus* species have been identified as potent extracellular enzyme producers that secrete enzymes like amylases, cellulases, lipases and proteases (Ferrari and Schmidt, 1993). Supplementation of these organisms may provide a source of these enzymes to the growing pig and aid the digestive process of various feed raw materials. This could lead to an improvement in nutrient utilisation and growth efficiency of the pig. Previous studies confirmed that there is a possibility of increasing the energy digestibility of pig feeds through dietary fibre digestion and fermentation mechanisms (Davis *et al.*, 2008; Lan *et al.*, 2017). An indirect secondary effect of fibre digestion from probiotic organisms could be the prebiotic effect that previously degraded fibre has on the large intestine, thus increasing cellulolytic fibre fermentation in the hindgut. This is confirmed by a study conducted by Jaworski *et al.* (2014a) where increased faecal volatile fatty acid levels were recorded when there was a probiotic added to the diet.

5.3 Feed intake

No significant differences were noted for ADFI of the pigs during this trial. Dietary energy caused a significantly increased feed intake in the reduced energy diets during the finisher phase. Although a higher feed intake was noticed for the reduced energy treatments, the ADG of the reduced energy groups was the same than that of the standard energy groups. It is possible that the high energy requirement during the finisher phase could have caused an upwards regulation of feed intake in the reduced energy treatments to satisfy energy needs. In general, pigs that are fed reduced energy diets will show increased feed intake to maintain a constant daily intake of energy until feed intake is limited by other factors. These factors include gut capacity or the concentration of other dietary components (Li & Patience, 2017). As an example, Tolhurst *et al.* (2012) reported that high fibre diets could supress appetite through secretion of additional hormones in the gut, thus lowering the dietary energy by increasing the dietary fibre level may not result in the expected feed intake compensation.

5.4 Feed Conversion Ratio (FCR)

Energy had a significant effect on the FCR of pigs during the weaner and grower 2 phase and a trend towards a significant effect (P=0.057) on FCR was also noted during the finisher phase. The FCR of pigs on the standard energy diets did not differ from those that received the reduced energy diets, except during the weaner phase where a significantly improved FCR was recorded for the standard energy diets (Treatment 2 and 4, diets with no added probiotic additive). The higher FCR recorded for the reduced energy diets in the weaner phase may be due to the limited ability to digest and utilise fibre sources as well as an underdeveloped microbiota and a growing demand for energy (Meng *et al.*, 2010; Patience, 2012; Fouhse *et al.*, 2016).

In the current trial the addition of a probiotic to both energy density diets only led to a marginally lower FCR when compared to the non-probiotic supplemented counterparts. Significant differences were noted between the standard energy with added probiotic and reduced energy with added probiotic (Treatment 1 and 3) in the weaner and finisher phases, with a trend in higher FCR also noted in the grower 2 phase for the reduced energy with added probiotic. The different modes of action that are described for probiotics may explain the lower FCR of the probiotic added treatments (Oelschlaeger, 2010; Brown, 2011; FAO, 2016; Liao & Nyachoti, 2017). The reduction in FCR with probiotic supplementation recorded in the weaner phase could most likely be due to the enhancement of the gut epithelial barrier integrity as well as overall gut health after weaning (Brown, 2011; Castillejos, 2018). Furthermore, gut health enhancement could also be due to a reduction in overall pathogenic populations as demonstrated by the study of Lan *et al.* (2017). Their study showed that probiotic supplementation led to increased faecal *Lactobacillus* counts and significantly decreased *E. coli* counts in weaned pigs. The finisher diets supplemented with probiotic may have resulted in FCR differences due to the increased efficiency of the standard energy diet with supplemented probiotic (Treatment 1) and the possible energy deficient state of the reduced energy diets during the finisher phase. This observation supports the increased ADFI of the reduced energy diets, without any effect on the ADG of the same period as discussed previously. These results are in agreement with the observation of Meng *et al.* (2010) that a higher nutrient density diet with supplemented probiotic could have optimised the gut microbiota balance, resulting in improved utilisation of nutrients.

Energy had a significant effect on cumulative FCR in the weaner, grower 1, grower 2, and finisher phases, with significant differences also seen between the standard energy and reduced energy diets (Treatment 2 and 4) from the weaner phase till the finisher phase. Feeding the higher energy diets resulted in a significantly better cumulative FCR. The cumulative FCR response to energy in the current trial is consistent with the results of Quiniou & Noblet (2012) who found that an increased inclusion of dietary NE leads to a reduction in ADFI as well as a reduction in the FCR of pigs. An important comparison in this trial was between the standard energy diet and the reduced energy diet with added probiotic (Treatment 2 and 3), in order to test if the probiotic was able to compensate for the reduction in energy (0.3 MJ/kg or 72 kcal/kg on NE level) through various modes of action. No significant differences were observed in cumulative FCR between these two treatments (Treatment 2 and 3), even though significant differences were recorded between the standard energy and reduced energy diets (Treatment 2 and 4). The results of this study corresponded with those of other researchers, where differences were seen in two energy density diets but no differences were recorded between a standard energy diet when compared to a reduced energy diet with added probiotic in terms of cumulative FCR (Meng *et al.*, 2010; Jørgensen *et al.*, 2016). This could indicate that the probiotic additive is able to utilise the NSP fraction of the diet and increase feed efficiency from maize and wheat bran sources over the entire growing period of pigs until slaugther. Alternatively, the probiotic has a nutrient contributing effect over long term exposure on cumulative FCR of pigs and that the effect could originate from an energy contribution

(Davis *et al.*, 2008; Meng *et al.*, 2010; Zhao & Kim, 2015; Jørgensen *et al.*, 2016) and/or an amino acid contribution from the fibre polysaccharide fraction (Bjerre *et al.*, 2016; Nørgaard *et al.*, 2016b; Torres-Pitarch *et al.*, 2016). Generally, long-term exposure of a probiotic on overall gut health and the secondary benefits coming from a healthy or stable gut environment could also influence the cumulative FCR as recorded between Treatment 2 and 3.

5.5 Slaughter parameters

The addition of a probiotic to the diet increased the carcass weight of supplemented animals significantly when compared to non-supplemented animals. Carcass weight of probiotic supplemented animals was very similar while the difference in carcass weight of non-supplemented animals was much larger. This gain in carcass weight is directly due to the increased growth rate witnessed in animals receiving the probiotic additive throughout this research trial.

As discussed previously, the increase in body weight gain and subsequent higher carcass weight are potentially a consequence of more efficient utilisation of the undigested feed fraction, a more mature microbiota population and a reduction in pathogenic gut organisms during and after weaning. These results are in agreement with a study conducted by Balasubramanian *et al.* (2016), where the addition of a probiotic supplement (of *Bacillus* origin) also increased carcass weight of test animals. Also worth noting is the gain in carcass weight in the reduced energy diet groups (Treatment 3 and 4). The addition of a probiotic additive to a reduced energy diet resulted in significantly heavier (+5.88 kg) carcasses. This result did not differ with the standard energy with added probiotic group (Treatment 1). The probiotic supplementation to a reduced energy diet, exceeded warm carcass weight performance of the standard energy diet up to a point where a tendacy towards a significant difference was recorded (P=0.077). The increased carcass weights as a result of probiotic supplementaion during the growing phase could be due to the optimisation of gut health and microbiota balance from an early age. This optimisation may aid in the digestive process by better utilisation of dietary nutriets over time (Alexopoulos *et al.*, 2004). In the present study back fat thickness, muscle thickness and lean meat percentage were similar between treatments.

CHAPTER 6
CONCLUSION

Many studies have been conducted over the past 30 years to substantiate the effect of probiotics in animal nutrition. Different organisms were researched with contrasting results and opinions from various researchers. The introduction of spore-forming probiotics instead of live probiotics in animal nutrition has changed the *in vivo* outcome of these additives for the better. Although we have a better understanding of the mode of action of probiotics today, a vast amount of knowledge must still be acquired through research. The complex interaction between probiotic organisms, other microbiota in the GIT and the host, as well as the change in dynamics of the host and the microbiota must be better understood to ensure constant and predictable results when using probiotic additives in the different life stages and ever changing GIT environment of the pig.

This study investigated whether a dual strain probiotic additive was able to utilise the undigested feed fraction to release additional nutrients to benefit the growing pig. A total of 0.3 MJ/kg or 72 kcal/kg on net energy level was reduced from a standard energy diet throughout five dietary phases that were formulated on commercially accepted dietary specifications. Soya oilcake and full fat soya were kept at the same inclusion level within dietary phases to limit any additional substrate, other than that from wheat bran and maize which was allowed to fluctuate to create diets with varying levels of energy.

Supplementing commercial pig diets with a dual strain probiotic additive significantly improved body weight and body weight gain from the grower 1 phase onwards until slaughter without affecting the feed intake of animals. Positive effects were noted in the carcass weight of probiotic supplemented vs. non supplemented animals. Probiotic supplemention of a reduced energy diet resulted in significantly larger carcasses when compared to the unsupplemented reduced energy group. The compounding effects of a beneficial microbiota balance from weaning, together with the various modes of action that the probiotic enables on the GIT over the entire growing period, most possibly contributed to the positive results seen on production parameters.

In this study however, the difference in energy density between the two diets was not large enough to enable a significant difference in most of the parameters measured, making it difficult to test if the probiotic had an energy or nutrient contributing effect. The NE of diets should have been lowered even more than the 0.3 MJ/kg or 72 kcal/kg to induce significant differences between different energy densities in order to test the effect of the probiotic between energy treatments. This may have been as a result of lower stress levels in the research trial facility when compared to commercial conditions. The reduced energy diets had adequate levels of energy to maintain decent animal performance until the finisher phase where energy limitations manifested in increased feed intake.

A very important comparison is that of the standard energy diet (Treatment 2) and the reduced energy diet with added probiotic (Treatment 3), as it was hypothesised that the probiotic additive should compensate for a lower energy density in the diet from the undigested feed fraction or NSP fraction. This effect was only recorded in the cumulative FCR of the standard energy diet (Treatment 2) and the reduced energy diet with added probiotic (Treatment 3), even though significant differences were recorded between the two dietary energy decities without added probiotics (Treatment 2 and 4). Probiotics therefore require longer periods of exposure within the GIT of the host as the cumulative effect on the micobiome, gut health and nutrient availability could result in sigificant efficiency gains over time. The possible reduction of pathogenic organisms in the gut and the shift in fermentation patterns from proteolytic to cellulolytic, could have a positive effect on gut health in general. One of the possible effects could be the release of nutrients (energy and/or protein) from the NSP fraction of pig diets, but requires further research to fully understand the effect of specific probiotic organisms on digestibility of fibrous raw materials. The selection of new, more effective and focused strains of probiotic organisms will unlock new possibilities as well as provide increased consistency in trial and commercial results. Probiotic organisms therefore continue to act as a promising feed additive to reduce “in feed” antibiotic use and increase feed efficiency in a holistic manner.

It can be concluded that the first alternative hypothesis of this trial could be accepted (H_1) as a probiotic supplement did improve feed efficiency as well as body weight and carcass weight of commercial pigs and pig diets.

The second null hypothesis (H_0) of this trial was that probiotic supplementation is unable to contribute 0.3 MJ/kg or 72 kcal/kg on net energy level from the dietary fibre fraction. The null hypothesis will be accepted, as it was not possible to test this hypothesis due to the difference in dietary energy levels that was not large enough to result in significant differences to compare between treatments in the research facility.

CHAPTER 7

CRITICAL REVIEW AND RECOMMENDATIONS

In the present trial, the inclusion of a dual strain probiotic additive had an effect on certain production parameters measured. Significantly higher body weights were recorded from the grower phase onwards till slaughter and heavier carcass weights were obtained. The addition of a probiotic could influence the cumulated FCR of supplemented pigs in reduced energy diets when compared to standard energy diets.

To evaluate the effect of a probiotic on nutrient digestibility and energy contribution, dietary energy was reduced by 0.3 MJ/kg or 72 kcal/kg on net energy level from a standard energy level. The effect of the lower energy was expected to result in performance differences between the standard and reduced energy diets to enable comparison with the inclusion of a probiotic. Unfortunately, no significant differences in basal energy levels were noted for most of the trial which made it difficult to evaluate the effect of the probiotic on nutrient digestibility. More profound differences in energy and/or protein densities need to be tested in order to quantify the effect of a probiotic on nutrient digestibility. Furthermore, the effect of the trial conditions could affect the outcome of results if commercial conditions and stress situations are not properly replicated or induced. Comparable commercial stress situations is crucial for measuring the effects that probiotics could have in scientific trials.

The probiotic additive used in this study showed potential as a viable alternative to increase growth rate and can form part of nutrition strategies to increase overall gut health and pig performance parameters. Since *Bacillus* organisms produce a range of enzymes that is dependent on the surrounding environment, the possibility of an amino acid contribution from the undigested or NSP fraction should not be ignored. In the present study, the focus has been placed on an energy contribution, but further research should be conducted in the simultaneous contribution of amino acids and energy from probiotic organisms from different feed ingredient types. Also, robust methods are required to accurately quantify the effect of probiotics on the digestion dynamics and nutrient availability within the GIT. The use of probiotic organisms in pig production systems should be seen as a long-term strategy to improve animal performance due to the cumulative and compounding effects from various modes of action, impacting feed efficiency, host health and balanced microbiome development.

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APPENDIX 1

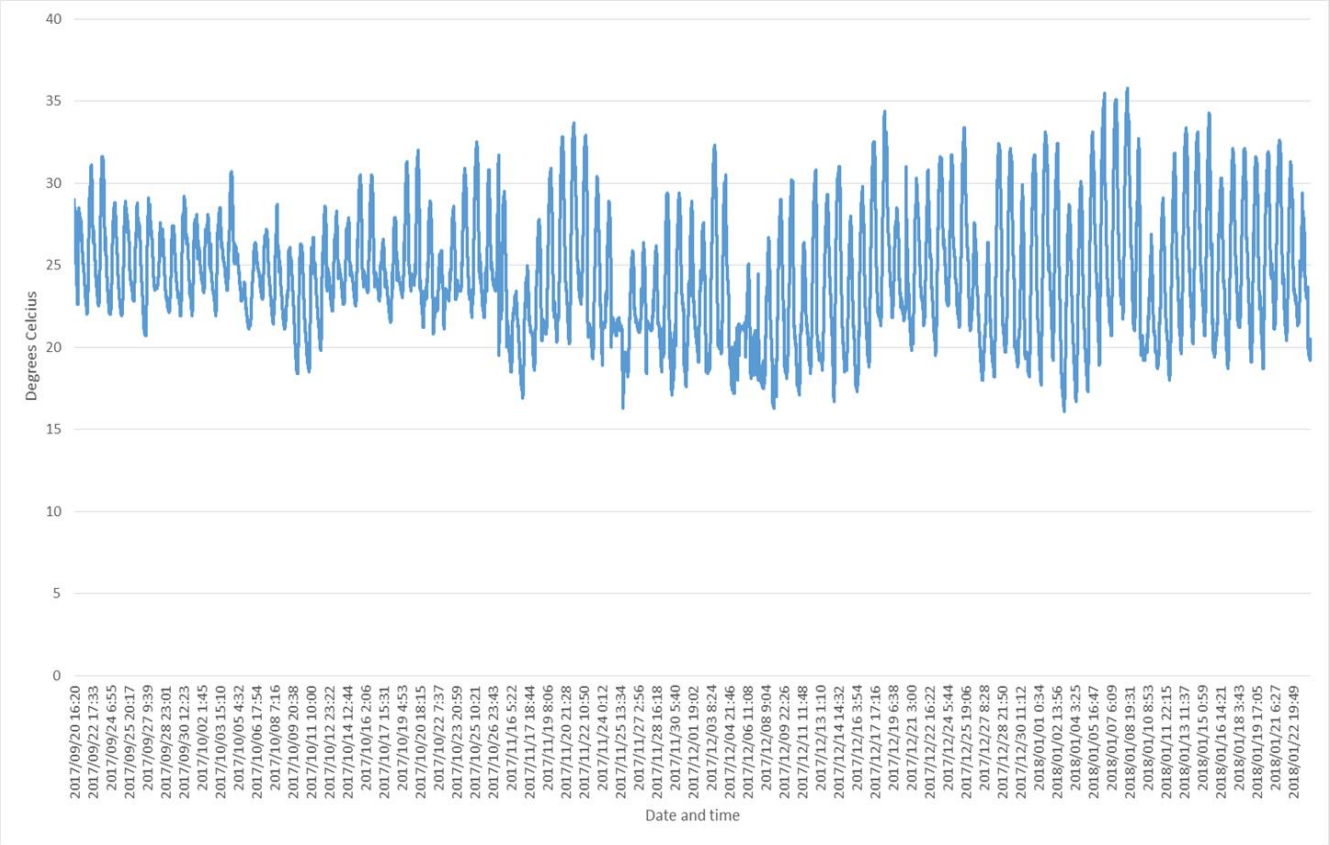

Figure 1. Minimum and maximum temperatures (°C) during the trial period