EFFECT OF PROBIOTIC AND LACTOFERRIN -SUPPLEMENTED DIETS ON DAILY GAIN, FEED INTAKE, FEED CONVERSION RATE, MEAN WEEKLY FAECAL SCORES, LYMPHOCYTE TO NEUTROPHIL RATIO, IMMUNITY, GENERAL HEALTH, AND HEMATOLOGICAL PARAMETERS IN WEANLING PIGS SUBJECTED TO AN IMMUNOLOGICAL CHALLENGE

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ABSTRACT

Background The digestive system of early weaned pigs is not fully developed and animals can be subjected to a post-weaning check, or lag period, which results in poor feed intake, weight gain, immunity and high diarrhoea cases and mortality. In addition, there is a depression of growth during an immune challenge that results in nutrient intake restriction and redirection to support the immune system. A shift from the unstable flora at weaning into a complex stable one would be achieved by diet manipulation. Different natural products (instead of hazardous antibiotics) are being tested to find their ability in improving pig health and performance.

The aim of this study was therefore to evaluate the effects of dietary supplementation with probiotics and lactoferrin on pigs' growth performance, haematological characteristics and general health. The weaned pigs from four different farms were mixed together upon arrival to place them in an immune challenging environment.

Results After 21 days post challenge/weaning, average daily feed intake, ADFI (404.64, 426.77, 423.63, 378.48 and 341.48 g/p/d for diet for diet A[control], B, C, D (probiotics) and E [lactoferrin], respectively) was significantly different in pigs that consumed the five diets ($p=0.0259$). Pigs that consumed diet B had 5.47% higher feed intake ($p<0.05$) than the controls, while those that received diet C consumed 4.69% more feed than the controls but this feed intake was not significantly different from that of the controls ($p>0.05$). The difference in feed intake between pigs in fed diet B and C was also not significant ($p>0.05$). Animals that were allocated to diet D and diet E (lactoferrin) consumed significantly less feed compared to the controls ($p<0.05$). Feed consumption was 6.47% and 15.61% lower ($p<0.05$) for pigs fed diet D and E, respectively, compared to the controls. Pigs that received diet D consumed significantly higher amounts of feed ($p<0.05$) than those fed diet E (lactoferrin).
In *conclusion*, in the first three weeks of life, or at times of stress such as weaning and / or immune challenge, a good probiotic (such as B or C) should produce a faster and more rapid response by increasing / stimulating feed intake so that body weight losses are quickly compensated. Feed intake is a factor that limit growth in weaned piglets. Weight is gained after the improvement in feed ingestion. Reduction in feed / energy

intake also reduces body weight. If feed (energy) intake is reduced, then, a good diet should stimulate quick repair of the gut and improve intestinal environment architecture and integrity. When feed consumption increases, the levels of digestive enzymes responsible for the breakdown of fats, starches and proteins increase. When feed intake is not increased or increased too late after weaning, bodyweight may not be compensated.

DEDICATION

I dedicate this thesis to my wife Oli, my sons: Sampa, Bwalya and Mambwe and daughter, Chimwemwe. This is one of the highest achievements for the benefit for all of us. MAY HIS NAME BE BLESSED AND GLORIFIED.

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LIST OF ABBREVIATIONS

BASO	Basophil cells
CH	Corpuscular haemoglobin constant
CHCM	Corpuscular haemoglobin concentration mean
CH	Corpuscular haemoglobin
CHOL	Cholesterol
Cu	Copper
DNA	Deoxyribose nucleic acid
EOSI	Eosinophil cells
ESR	Erythrocyte sedimentation rate
HCT	Haematocrit level
HGB	Haemoglobin concentration
LYMPH	Lymphocyte cells
(L/N ratio	Lymphocyte to neutrophil ratio
MCH	Mean cell haemoglobin (or mean erytrocyte haemoglobin content)
MCHC	Mean corpuscular haemoglobin concentration (or mean erythrocyte haemoglobin concentration)
MCV	Mean cell volume (or mean erythrocyte volume)
M	Molar
MONO	Monocyte cells
NEUT	Neutrophil cells
ppb	Parts per billion
RBC	Red blood cells
RDW	Red blood cell distribution width (or erythrocyte distribution width)
SAS	Statistical Analysis System
TG	Triglyceride
USDA	United States Department of Agriculture
WBC	White cells

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INTRODUCTION

As pointed out by McDonald *et al.* (2002), World demand for meat on an absolute basis (i.e allowing population growth) is predicted to increase rapidly; by 0.6 per cent per year in developed countries and by 4.1 per cent per year in developing countries. In general, livestock productivity can be increased through good nutrition, improved management and disease control and genetic improvement (Antipas & Weber, 2003, Blecha & Charley, 1990). One of the management tools used to reduce disease burden and increase animal performance is antibiotics (Walton, 1979, 2001; Barnett *et al.*, 1989; Cromwell, 2002; Kostantinov *et al.* 2004).

Antibiotics have been used in the animal industry to improve performance (Walton, 2001). However, public concerns exist about potential risks such as bacterial resistance and allergenic effects in consumers of animal products. As a result, Sweden and Denmark prohibited the use of prophylactic antibiotics in the mid 1990's and this was followed by the European Union (Williams & Heymann, 1998; Witte, 1998; Yu *et al.* 2004; Close, 2000; Cullen *et al.*, 2000; Wenk, 2000; Barnett *et al.*, 1989; Jensen, 1998; Bager *et al.*, 2000; Adjiri-Awere & van Lunen, 2005). Prohibition of antibiotic use seems reasonable and desirable to consumers (Witte, 1998; Hiss & Sauerwein, 2003; Jensen, 1998; Lopez, 2000). However, the banning of antibiotics may lead to higher stress, mortality and increase the number of days to marketing in weaned pigs (Walton, 2001). To increase productivity of sows (and / or farrowing index), piglets are weaned early (at about four weeks or earlier instead of about 8-12 weeks of age). Early weaned pigs can be stressed by change of feed and of psychological, social environment and dietary stress (Dantzer & Mormede, 1983; Morrow-Tesch & Anderson, 1994; Lombardi *et al.*, 2005; Mahan & Lepine, 1991; Lalles *et al.*, 2004). Since their digestive system is not fully developed, they can encounter a post-weaning check, or growth lag period which is characterised by low feed (energy) intake, diarrhoea and low weight gain loss (Risley *et al.*, 1988; Barnett *et al.*, 1989; Spreeuwenberg *et al.*, 2001; Kostantinov *et al.* 2004; Roth & Kirchgessner, 1998; Aherne *et al.* 1982; Mahan & Lepine, 1991; Funderburke & Seerley, 1990; Bosi, 2000). After the ban of antibiotics in Sweden, post-weaning mortality rates increased by 1.5% and piglets took 6 days longer to reach 25kg target weight for age

(Robertsson & Lundeheim, 1994 (cited in Adjiri-Awere & van Lunen, 2005)). This evidence shows that lack of antibiotics creates serious negative actions. To correct this, zinc oxide was used and post weaning mortality declined from 1.5% (reported above) to 1%, and time needed to reach the 25kg bodyweight reduced from 6 to 4.5 days (Wierup, 1999, cited in Adjiri-Awere & van Lunen, 2005).

Researchers in the animal and feed industry are now trialling some alternatives and natural products, ranging from chemicals and organic acids to biological products such as probiotics. These products have properties similar to antibiotics, as they improve growth and suppress pathogens (Jensen, 1998). Antibiotics can be used in known amounts to target certain pathogens. Probiotics, on the other hand, produce variable and unpredictable effects and their mode of action is not fully understood. Natural products, such as probiotics and lactoferrin, have been found to improve growth, establish a prophylactic barrier against gastrointestinal disorders. These effects have been greater in young animals and those under stress (Jensen, 1998). The integrity of the gut mucosa is a prerequisite to reduce the entry of antigens (Bosi, 2000). The mucosa of the gastrointestinal tract is the first line of contact with pathogens that are ingested. The components of the gut mucosal barrier viz non-immunological (such as lactoferrin) and immunological are however disturbed at weaning (Bosi, 2000). Reduced feed intake in the weaned pig causes villous atrophy (Bosi, 2000). A lowered supply of milk (Kelly *et al.*, 1991) or a restricted ingestion of dry feed (Pluske *et al.*, 1996) reduce the height of villous on the fifth day post weaning.

A good probiotic must not only enhance growth performance but must also be devoid of adverse effects (Bernardeau *et al.,* 2002). Probiotics such as lactic acid bacteria (LAB) have been incorporated in feeds for many years and have been classified as safe (Fuller, 1992). These are therefore referred to as "Generally Recognized As Safe": GRAS (Fuller, 1992; Bernardeau *et al.*, 2002). In addition to probiotics, other natural products such as lactoferrins are said to have an effect on performance and on cellular immune function of ruminants (Wong *et al.*, 1997).

The aim of this project was to look at the effects of five different diets (a control or basal diet and four supplemented diets) on average daily gain, ADG, average daily

feed intake, ADFI, feed conversion ratio, FCR, body weight, mean weekly faecal score (MWFS), stress factors (or lymphocyte to neutrophil) (L/N) ratio and general health performance in weanling pigs. Other studies may have looked at different probiotics, environment, species, concentration etc, but not many have looked at using lactoferrin (diet E) in piglet diets. The three probiotics (B, C and D) used in this study are not known (names withheld by the sponsor) except for lactoferrin (a biologically active protein) in diet E and therefore a wide literature search has been made in order to match the effects of natural products with the ones used in the current study.

Chapter 1

1. LITERATURE REVIEW

Probiotics are claimed to have many effects on the host. Among these effects are: improvement of performance, immune system and general health (Lopez, 2000). In this literature review it is therefore important to give brief summaries of what a probiotic is, and some understanding of effects of probiotics and other natural products on performance of animals.

1.1 Probiotics

1.1.1 History of claims on probiotic use

The benefits and use of probiotics such as lactic acid bacteria to cure gastrointestinal ailments was known since time immemorial. In Genesis 18:8 of the Holy Bible, it can be read that Abraham owed his longevity to the consumption of fermented milk. Similarly, European interest in the gut health benefits of yoghurts containing "beneficial bacteria" began in the early 1900s, and was indorsed by Metchinkoff and Tissier at the Pasteur Institute (Cummings *et al.*, 2004). Metchinikoff in 1907 claimed that consumption of yogurt (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) results in a decline of harmful microorganisms but *Lactobacillus bulgaricus* were later shown that they did not survive in the gut (Schrezenmeir & de Vrese, 2001). European commercialisation of specific yoghurts based on their gut health was initiated as early as 1920 by Carasso *et al.*, as cited in Cummings *et al.*, 2004. Consumption of this product in Europe is now greater than 15 kg capita per year and the amounts are rising (Cummings *et al.*, 2004).

1.1.2 Definition of probiotic

The term probiotic means 'for life' in Greek language. Lilly and Stillwell in 1965 were the first to use the word probiotic to describe "substances secreted by one microorganism which stimulates the growth of another" and thus was different with the term antibiotic (Lin, 2002) although Kornegey & Risley (1996) reported that the administration of "beneficial organisms" to animals started in the 1920's and the name "probiotics" (in the sense it is used today) was introduced by Parker (1974) when the

production of bacterial feed supplements began on a commercial scale and this is in agreement to Schrezenmeir and de Vrese (2001).

1.1.3 Revision of definition

The definition of a probiotic has had a lot of words added, and subtracted or broadened and narrowed, with some fine tuning and refining. A general definition is: a probiotic is a live culture of microorganisms, such as lactic acid bacteria (LAB), that exerts a beneficial effect on the host by improving the indigenous microbial balance (Fuller, 1992). Probiotics as applied to human, are defined as live microrganisms which, when administered in adequate numbers, confer a health benefit on the host (FAO/WHO, 2001 (as cited by Casey *et al.* (2004)). Another definition for probiotics is "living microorganisms, which upon ingestion in certain numbers, exert health benefits beyond inherent basic nutrition" (Guarner and Schaafsma, 1998 (as cited in Adjiri-Awere & van Lunen, 2005)). Some workers have limited the definition to milk and others on health and nutrition benefit which again included antibiotic but a more improved, revised and broadened definition with respect to host and habitat which does not limit proposed health effects and excludes nutrition is: 'a probiotic is a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonisation) in a compartment of the host and, by that, exert beneficial health effects in the host' (Schrezenmeir & de Vrese, 2001).

Furthermore, other workers have found that some probiotics such as lactobacillus cultures still maintain their probiotic effect wether dead or alive (Bernardeau *et al.*, 2002; Salminen *et al.*, 1999). With these findings, the additional definition proposed by Salminen *et al.* (1999) is "probiotics are microbial cell preparations or components of microbial cells that have a beneficial effect on the health and well being of the host".

1.1.4 Characteristics of effective probiotics

Probiotics are characterised as living microorganisms. A good probiotic should be able to escape degradation in the gut (Yu *et al.*, 2004; Adjiri-Awere & van Lunen, 2005), improve growth and feed efficiency and leave no tissue residues or cause mutations (Atherton & Rubbins, 1987; Lopez, 2000; Stavric, 1992).

On the other hand, antibiotics are characterised as pure chemical compounds. They are absorbed in the digestive tract. They also improve growth and feed efficiency but they leave residue in tissue and may cause mutation (and resistance) in other microorganisms. The general mode of action of probiotics is that they produce acids, reduce pH and compete with, or discourage growth of pathogenic microorganisms. They posses localised antimicrobial activity and proliferate in the digestive tract and compete for nutrients and space with the pathogenic bacteria whereas antibiotics improve the host's performance by blocking living cell's DNA, RNA or protein synthesis and they have a broad spectrum of activity (Lopez, 2000). Good natural products such as probiotics must not only enhance growth performance but must also not cause adverse side effects (Bernardeau *et al.*, 2002). Products such as lactic acid bacteria (LAB) have been used in animal feeds for many decades and have been classified as safe (Fuller, 1992). These products are said to have a "Generally Recognized As Safe": GRAS status (Fuller, 1992; Bernardeau *et al.*, 2002).

1.1.5 Development and production of probiotics

Production of probiotics starts with identifying strains that can proliferate in the gut and be recovered in the same numbers in the faeces of the host. As described by Stavric (992) this starts with the sourcing of isolates. The media for isolation is then identified and this is followed by characterization and storage of isolates. The experimental protocol is then chosen and the method of preparation of defined mixture is established. Selection of isolates from a large number of partially identified strains is made and this should match and be representative of all major groups found in the collected bacteria in the gut. These should be able to protect the host from increasing challenge by pathogens. This is then followed by deletion experiments and adheration and determining the mechanisms such as hydrophobic interactions or differences in surface charges, that play a role in the adherence of microorganisms (Gibbons *et al.*, 1983 (cited in Stavric, 1992)). In summary, the prospects for developing a treatment comparable to undefined culture, in efficacy and stability are not promising, despite large numbers of researches conducted. A better understanding of the mode of protection and factors involved would ease the manipulation of caecal flora on a more scientific basis (Stavric, 1992). Interestingly, dietary condition can affect the development of probiotics (Bosi, 2000) and may be, their effect. For example the

faecal numbers of *Bifidobacteria* were higher in pigs fed *Bifibobacterium longum* with high amylase cornstarch than with a low amylase cornstarch (Brown *et al.*, 1997 as cited in Bosi, 2000)).

1.1.6 Antibiotics and their use

Antibiotics and antimicrobials have been used to the furthest extent as additives in the rations of poultry and pigs for more than four decades (Adjiri-Awere & van Lunen, 2005). Antibiotics are substances that can destructively affect bacteria either by interfering with their growth, metabolism or actually killing them *in situ*. Antibiotics are also used to control bacteria that reduce the physiological or metabolic performance of animals. Antibiotics are included to the feed of animals because very large number of them can be given for a particular treatment at the same time, which lessens the trauma of handling that would be required for individual dosing and in the herd situation. It is also important to get all the animals at risk treated in an identical manner with the same concoction at the same time (especially animals housed in large numbers such as pigs and poultry) (Walton, 1979; 2001). Antibiotics are incorporated in food animals for: 1) therapy of disease and prevent deaths, 2) preventing the establishment of disease, 3) reducing pain, 4) circumvent secondary bacterial infection, 5) avoid development of an epidemic, 6) establish the gut flora and so enable the animal to cope with periodic changes of dietary ingredients and 7) improve physiological and metabolic performance (Walton, 2001; Nunes & Guggenbuhl. 1998; Hays, 1978; Stavric, 1992).

1.1.7 Benefits of antibiotics

The benefits of antibiotic use for humans include: 1) less expensive food, 2) food that is more acceptable to the needs of the consumer, such as less fat, 3) pigs that are managed free of persistent diseases, 4) more carcass meat with no trace of disease, and 5) shortened growing period to market point and therefore meat will be soft. The benefits to the animal are: 1) rapid healing of bacterial diseases, 2) lowering of low grade ailments, 3) alleviation of pain, 4) capacity to cope with constantly fluctuating dietary ingredients without the development of intestinal problems and 5) lowering of persistent toxicity by avoiding the growth of intestinal bacteria that produce a range of toxic substances such as ammonia and monoamines (Walton, 2001; Cromwell, 2002;

Adjiri-Awere & van Lunen, 2005); Growth promoting effects of antibiotics are not clearly understood, although the increase in growth rate might be as a result of improved gut health, nutrient utilization and improved conversion efficiency (Visek, 1978; Adjiri-Awere & van Lunen, 2005). The beneficial effects of subtherapeutic antibiotic use are found to be greatest in herds raised in sub-optimum sanitary conditions (Zimmerman, 1986) and have little effect on healthy animals reared in "exceptionally clean' conditions (Taylor, 1999; Adjiri-Awere & van Lunen, 2005).

In pig production, during the lactation period, and few days before and after weaning, deaths can range from 10 to 20 % as a result of stress factors (Piloto *et al.*, 2000 (as cited in Bocourt *et al.*, 2004)). Piglets weaned earlier (at 21 to 28 days of age) are exposed to adverse changes in environmental, social and dietary regime, and are often stunted or have retarded live weight gain, lowered feed ingestion and are easily predisposed to diarrhoea (Gabert & Sauer, 1994). These factors, among others, can affect the formation of a normal gastrointestinal flora and change its balance (Vandelle, *et al.*, 1990; Bocourt *et al.*, 2004), which incite greater incidence of diseases and higher rate of deaths, as well as the reduction in the production levels (Mosson, 2001; Swientek 2003; Bocourt *et al.*, 2004). Newly born and young piglets can easily be exposed to stress, which is, in part, due to their gastrointestinal tract (GIT) being sterile at birth and they do not have sufficient acidification ability in their stomach (Vandelle *et al.*, 1990; Bocourt *et al.*, 2004). In addition, they are born agammaglobulinemic and the thermoregulating and enzymatic mechanism of their digestive system is poor (Sainsbury, 1993; Bocourt *et al.*, 2004). Bocourt *et al.* (2004) reported that mortality in suckling pigs can be as high as 20% and as many as 41.5% of these deaths could be due to diarrheoic diseases. Out of these, 25.5% are produced by *Escherichia coli* (Brizuela, 2003 (as cited in Bocourt *et al.*, (2004)). In recent decades, the method to prevent diseases and increase feeding efficiency was the use of antibiotics as feed additives.

Hardy (1999) summarised seven reports showing that dietary antimicrobial agents improved digestibility of energy, nitrogen and phoshorus in pigs by 5.1, 1.8 and 3.4%, respectively. This is also in agreement with Chen *et al.* (2005) who reported that antibiotics increase growth rate as a result of improved gut health, nutrient utilisation

and improved feed conversion efficiency, although the mechanisms involved are not fully understood (Adjiri-Awere & van Lunen, 2005). Similarly, other workers have reported the same benefits of growth-promoting antibiotics in animals, including reproductive efficiency (Yu *et al.*, 2004) and decreased excretion of nitrogen (Close, 2000).

1.1.8 Disadvantages of antibiotics and reason for ban

Despite their use in low levels (sub-therapeutic) as growth promoters, increasing feed efficiency, reducing mortality and increasing reproductive efficiency, in recent years, their use has much disputed due to the latent risk of other bacteria securing resistance to particular antibiotics and the damaging effects, such as allergies, that this might have on human health (Adjiri-Awere & van Lunen, 2005). These environmental and consumer bones of contention are now very high on the political agenda of many governments, and the lawful use of antimicrobials and hormones in intensively-housed food animals is coming increasingly into question by sensationalistic, welfare, economic and political organisations alike (Walton, 2001; Adjiri-Awere & van Lunen, 2005).

It has also been reported that prolonged inclusion of antibiotics into pig feeds as growth promoters, fail to produce a benefit in some 20% of cases and probably do not provide an economic return in a further 20% (Rosen, 1992; Stewart & Chesson, 1993). While supporting the use of antibiotics to a small extent (providing they are not used at all times), Walton (2001) reported that good management practices (which includes appropriate use of antibiotics and disinfectants) can increase performance, especially when outbreaks are foreseen. But he blamed the non-farming public, and often the non-specialist press, who hold onto and disseminate the incorrect opinion that all pigs and poultry are reared from birth on feed containing antibiotics which are also used for the treatment of disease in man and in animals (Walton, 2001). Other workers have reported that, despite increasing performance in some cases, it is also been reported that antibiotics can interfere in the establishment of a normal gastrointestinal flora and alter its balance (Vandelle *et al.*, 1990; Bocourt *et al.*, 2004) which incites greater incidence of diseases and higher death rates, as well as reduction in production levels (Mosson, 2001; Swientek, 2003; Bocourt *et al.*, 2004). Bocourt *et al.*, (2004), reported

that antibiotics as feed additives have been proved to affect negatively the eubiosis of the gastrointestinal tract. Similar to Adjiri-Awere & van Lunen (1996), they reported that besides, the bacteria become resistant to them and Bocourt *et al.*, (2004) added that, in some cases, there are antimicrobial residue present in meat, milk and other products of animal origin.

The Swann Committee Report (1969) (as cited in Awere Adjiri-Awere & van Lunen, 1996), was the first to recommend that the use of sub-therapeutic levels of antibiotics for growth promotion and disease prophylaxis could increase the risk of bacteria securing resistance to particular antibiotics, and thus negatively impact the medical community's ability to combat human bacteria diseases. Since that time, others have reported it repeatedly to emphasize the concern.

1.1.9 Pig growth and appropriate times to use antibiotics or probiotic

1.1.9.1 Pig growth in general

Pig growth in general has been reviewed by numerous researchers such as Pearson and Dutson (1991), Cummings *et al.* (2004), Lalles *et al.* (2004).

1.1.9.2 The piglet after weaning

Sources of stress at weaning may be psychological (Funderburke & Seerly, 1990), nutritional (Funderburke & Seerly, 1990; Park *et al.*, 1986; 1984; Barnett., 1989), environmental (Crenshaw *et al.*, 1986; Barnett, 1989; 1980; Funderburke & Seerly, 1990), too early weaning, or low weaning weight (Mahan & Lepine, 1991).

Sources of stress at weaning may also be because of too early weaning, low weaning weight and these can interfere with gut development (Crenshaw *et al.*, 1986; Barnett *et al.*, 1989; Mahan & Lepine, 1991; Dantzer & Mormede, 1981 (cited in Funderburke & Seerly, 1990); Lalles *et al.*, 2004; Cummings *et al.*, 2003). Intestinal alterations often seen post-weaning in piglets include changes in villus/crypt morphology and brush border enzyme activity, and implication of enteric pathogens such as *E. coli* and rotaviruss (Pluske *et al.*, 1997; Lalles *et al.* 2004).

The integrity of the gut mucosa is a prerequisite to lower the entry of pathogens (Bosi, 2000). The components of the gut mucosal barrier (non immunological and immunological) are, however, disturbed during stress such as at weaning. Examples of components of the gut mucosa are given in Table 1. Lowered feed ingestion in the weaned pig causes villous to reduce in size (Bosi, 2000). A lowered supply of milk (Kelly *et al.,* 1991) or a restricted ingestion of dry feed (Pluske *et al*., 1996) reduce the height of villous on the fifth day post weaning. Inadequate feed intake in weaned piglets may result into intestinal inflammation and affect intestinal morphology. In addition, the antigenicity of the diet is another factor (Bosi, 2000). The defense against pathogens is integrated by the endogenous secretion of many antimicrobial components: hydrochloric acid, salivary lysozyme, defensins, lactoferrins, mucous secretion, bile salts (see Table 1). Some of these, however, the mechanisms of control of secretion are not adequately known to increase their production by dietary means (Bosi, 2000). As feed intake increases, the levels of digestive enzymes responsible for the breakdown of starches, proteins and fats increase. Therefore, making the animals to consume more feed shortly after weaning is very important (Lombardi *et al.*, 2005). As reported by Hiss and Sauerwein, 2003, reduction of pathogen load by β-glucan in the systemic effect medaited by the central nervous system might contribute to increased feed intake. This is also in agreement with Langhans and Hrupka, 1999 (cited in Hiss and Sauerwein, 2003).

As reported earlier, during the lactation period and for a few days pre- and post-weaning, deaths can range from 10 to 20 % as a result of stress factors (Piloto *et al*., 2000 (cited in Bocourt *et al*., 2000)). The cause of diarrhoea may vary, and the impact on health and well-being can range from slight discomfort to severe malnutrition and death in humans (Brown, 1994; Lin, 2000; Tungthanathanich, 1994; Kendiah, 1999).

Table 1: Components of the gut mucosal barrier in the piglet

Non-immunological	immunological
Mechanical	Local
Healthy enterocytes	Gut-associated lymphoid tissue
Cell turnover	
Tight junction	Intra-epithelial lymphocytes
Normal motility	Mesenteric lymph nodes
Chemical	
Gastric acidity	Aggregates in the lamina propria
Salivary lysozyme	Peyer's patches
Defensins	Secretory IgA:
Lactoferrin	endogenous
	maternal
Lactoferrin	
Mucous secretion	Systemaic
Bile salts	
Bacteriological	Circulatory lymphocytes
Aerobic and anerobic microorganisms	Hepatic Kupffer cells

Source: Bosi, 2000 p.279

1.1.9.3 The pig at weaning

Pig production can be improved by shortening the lactation period hence early weaning (3 to 4 weeks instead of about 8 to 12 weeks). But this can also bring some problems. Some of these problems are highlighted below. At weaning there is (1) reduced lactate (Pluske *et al.*, 1997; Lalles *et al.* 2004). Enteric infections after weaning further depress enzyme activity (Mroz *et al.*, 2003; Lalles *et al.* 2004); (2) reduced absorption (Lalles *et al.*, 2004). There is also (3) production of intestinal cytokines which are products that occur naturally and are capable of causing inflammations either at a reduced rate or to destroy the malignant cells. Pro-inflammatory cytokines IL-1β, IL-6 and TNF-α are usually the first harmonizers produced in reaction to tissue damage and are said to influence intestinal epithelial permeability and ion transport (McKay & Baird, 1999, (cited in Lalles *et al.*, 2004)).

(4) Protein and amino acid metabolism - between the time the pig is born and 14 days after weaning, the weight of the gastrointestinal tract of the piglet increases from 2 to 6% of body weight (Burrin & Stoll, 2003; Lalles *et al.*, 2004). Briefly after weaning, anorexia is evident and is followed by a reduction in small intestine protein and DNA mass, especially in the area of the small intestines. When anorexia is overcome, feed consumption increases and this is followed by an increase in small intestine weight that exceeds the rate of bodyweight gain, showing the greater important of the intestinal tissues for growth (Seve *et al.*, 1986, (cited in Lalles *et al.*, 2004)). (5) Integration of gut patho-physiological data- there is an interaction between feed intake, body growth and intestinal villus height trans- and paracellular transport correspond positively, and villus height negatively, with CD8+ T lymphocyte density, thus associating inflammation to morphological and functional changes during weaning (Lalles *et al.*, 2004) and (6) alterations in gut structure and function- low feed intake shortly after weaning in pigs is said to be the leading aetiological factor in gut disorders (Pluske *et al.*, 1997; Lalles *et al.*, 2004). Improvement of gut structure, integrity and function would be achieved through use of means that can stimulate postweaning feed intake, such as an adequate diet. Milk products, such as skim milk powder, have a favourable effect on feed intake, growth performance, feed efficiency and health in piglets because of high digestibility of proteins and energy (Thacker, 1999 (cited in Lalles *et al.*, 2004)). Spray-dried plasma (SDP) has shown some improvement in growth performance as a result of increased feed intake (van Dijk *et al.*, 2003; Lalles *et al.*, 2004). This improvement, is not observed in the presence of ingredients of plant origin (Lalles *et al.*, 2004). Recent studies have proved that spray-dried plasma is also protective during the first week after weaning when added together with other nutrients into drinking water (Steidinger *et al.*, 2002, (cited in Lalles *et al.* 2004)), this decreased the incidence and severity of post weaning diarrhoea and intestinal damage. Other studies with SDP, however, did not show improved responses to *E.coli* challenge (van Dijk *et al.* 2002; Lalles *et al.*, 2004) or reported immune over-response and increased intestinal damage following a lipopolysaccharide challenge (Tochette *et al.*, 2000; Lalles *et al.*, 2004).

Weaning, change of diet and use of antibiotics can be stressful in an animal and can change the eubiosis of the gut milieu (Kelly, 1998). At weaning, piglets have poorly developed non-immune and immune barrier functions.

As reported earlier, pig production can be improved by shortening the lactation period, i.e early weaning. In summary, the early weaned pigs can be affected by several stresses as explained above, through too early weaning or at low weaning weight (Mahan & Lepine, 1991, Lalles *et al.*, 2004). Low feed intake (<100 g/p/d preweaning) may sensitise the pig to antigens in certain feed ingredients (Newby *et al.*, 1983; Barnett *et al.,* 1989). Exposure of sensitized pigs to the dietary antigens at weaning may result in immune responses that damage the lining of the intestinal tract. The resulting post-weaning scours may be caused by malabsorption and loss of electrolytes, by which, in conjuction with depressed appetite, result in poor performance (Barnett *et al.,* 1989). Such dietary hypersensitivity may be transient because pigs recover from this phenomenon in less than 2 weeks postweaning. Longer exposure to, or greater consumption of, the antigenic proteins may induce tolerance to these antigens.

The skill of the manager aims to prevent young piglets from picking up a variety of diseases during this period and he is aided in this by the use of management aids, including disinfectants and antimicrobials (Walton, 2001; Nunes & Guggenbuhl, 1998). Supplementation of diets with antibiotics to prevent diarrhoea post weaning may result in selection of resistant strains of disease causing bacteria. Therefore, interest in "natural" feed additives, such as probiotics, organic acids and minerals has developed (Gabert & Sauer, 1994).

1.1.10 Effects of antibiotics ban

Livestock development has been rapid because some Governments have given subsidies, both hidden and transparent to develop intensive methods of farming so as to produce cheaper food. Intensively managed animals suffer less from disease due to improved husbandry. But after achieving this goal, the same Governments are now advocating stricter methods of production. Many supermarkets are now confusing management systems by laying down requirements, sometimes unreasonably, for the husbandry and management of the food animal that they intend to purchase from producers and sell through their retail outlets. These requirements focus especially on welfare and reducing the use of in-feed antimicrobial substances, including performance boosters. The supermarkets are trying to ensure that any meat, eggs or milk they sell will be produced in a way acceptable to the consumer. Also, they

consider that because of the controlled methods of production, these foods will not contain any antimicrobial substances that would place the consumer at risk thus improving the image of their sales. However, it must be evident that most systems of animal production that incorporate judicial use of antimicrobials can maintain output at a reasonable cost, which can be passed on to the supermarket and thence to the consumer (Walton, 2001). This is similar to Prescott (2000) who estimated that the forbidding of subtherapeutic use of antibiotics in food animals would add $5 to $10 a year to the cost of food for each American citizen.

1.1.11 Alternatives to antibiotics

Since the ban might result in reduced performance, this has driven animal nutritionists and producers to devise natural substitutes, such as probiotics, for commercial pig farms to reduce the problem of higher death rates and reduced growth performance following this restriction (Link *et al.*, 2005; Yu *et al*, 2004; Adjiri-Awere & van Lunen, 2005).

Denmark announced, as of November 2002, a complete withdrawal of growth promoters by 2006 (Fischer-Boel, 2002 as cited in Awere Adjiri-Awere & van Lunen, 2005). Despite the current interest in getting rid of subtherapeutic antibiotic use in animal production, there may be a risk that such a lowering or removal would have negative effects on the animal welfare, nutrient utilisation, manure production and economic sustainability. Close (2000) indicated that in-feed antibiotics for pigs produce a 5- to 10-fold return because production efficiency is raised.

In the EU two Bacillus products are licenced for animal use, BioPlus® and Toyocerin®. Toyocerin contains a strain of *B. cereus* var toyoi that has been deemed safe for animal use because of its failure to produce enterotoxins and its failure to transfer antibiotic (Hong, 2005).

1.1.12 Way forward

As reported earlier, consumer groups and the European Union (EU) have decided to forbid all antibiotics used sub-therapeutically in farm animal feed, driving animal nutritionists and producers to devise natural alternatives such as probiotics for

commercial pig farms to reduce the problem of higher death rates and reduced growth performances following this block.

Recently, studies relating to the health benefits and therapeutic effects of some harmless live microroganisms has become one of the hottest topics in nutritional sciences (Qu, 2001).
Technologies in this area are rapidly developing, based on new biotechnology. Enzymes, yeasts and live bacteria and their metabolites are involved in these investigations.

There is also need to develop species resistant to diseases (Tizard, 2000).
Justification for health claims comes mainly from animal studies. These have shown that germ-free (gnotobiotic) animals, or animals whose gut microflora have been perturbed by antibiotics are more susceptible to disease, and resistance can be restored by oral administration of faecal suspension from healthy individuals, a routine taken by the USDA to reduce salmonellosis infection in commercially reared chicks. The organisms used in probiotics are known to produce antimicrobial substances that might affect the colonic microflora balance (Scheinbach, 1998).

On the other hand, probiotics supplementation to piglets to improve growth performance has shown different results, and the precise mode of action through which probiotics exert their positive influence is not fully understood, although the mechanisms by which they improve health include:
-reduction in the amount of viable microorganisms by-

- Production antimicrobial compounds such as acids, peroxides or bacteriocins bactericidal to groups that negatively impact health;
- Competition with disease causal organisms
- Competition with disease causal organisms for mucosal binding sites;
- Competition for substrates

-stimulation of the immune system through increase in the concentration of IgG which increases the number of antibodies and macrophage activity (Scheinbach, 1998; Lopez, 2000).

-modification of the microbial metabolism through increased enzymatic activity (e.g. beta-galactosidase which decreases lactose intolerance) and decrease in enzymatic activity (e.g. beta-glucuronidase and nitroreductase) (Lopez, 2000).

Other benefits include reduction of:
-lactose intolerance
-cholesterol
-cancer (Lopez, 2000; Srivanasan, 2005).

1.1.13 Methods to improve performance after the ban

In Sweden, the performance of animals declined after the ban of in-feed antibiotics as stated above. Initially, post-weaning mortality rates in that country worsened / increased by 1.5% and days to 25 kg increased by 6 days (Robertsson and Lundeheim, 1994 (cited in Adjiri-Awere & van Lunen, 2005)) and this is also in agreement with Goransson, 2001 and Walton, 2001 who also added that the number of diarrhoea cases doubled.

Following the prohibition of in-feed antibiotics in the EU, the effectiveness of some of the available alternatives, such as probiotics (Pollman *et al.*, 1980) has evaluated. Enzymes, such as microbial phytase, has been found to release phytate-bound phosphorus in ingredients of plant origin and improve utilisation of phoshorus and amino acids in broiler starter feeds (Ravindran *et al.* 2006; Patridge and Hazzledine, 1997). Fermented dairy products, such as yoghurt and fruit juices, (Cummings *et al.*, 2004); fermentable carbohydrates, such as sugar beet pulp, and fructooligo-saccharides increase the number of beneficial lactobacillus bacteria. They also stimulate higher bacterial diversity and more rapid stabilisation of the bacterial community, resulting in improved health but not necessarily growth (Konstantinov *et al.*, 2003), Mannan oligosaccharides (MOS) inhibit the colonisation of some strains of bacteria in the intestinal tract, such as *E. coli.* MOS acts as a recptor for E. coli, binding the microbe (Davis *et al.*, 2002). Bacterial adherence results in the alteration of the intestinal microflora, and may be the mechanism by which MOS improves growth performance in pigs (Davis *et al.*, 2002). Similar prebiotics include dietary fibres (non-starch polysaccharide) such as inulin, that improve gut health, bowel habit

and satiety, and synbiotics such as bakery, cereal products (Cumming *et al.*, 2004) Average daily gain and feed intake is improved by using dietary β-glucan (Hiss & Saurwein, 2003) and the improvement was higher on farms with low hygienic status (Decuypere *et al.*, 1998; Hiss & Sauerwein, 2003).

Products such as copper sulphate improves health and growth performance (Davis et al., 2002), due to its antimicrobial effect which improve the gut. Both Cu and mannanologosaccharides (MOS) are believed to alter lymphocyte response *in vitro* (Davis *et al.,* 2002). However, feeding high levels can result in more Cu in the manure and pose an environmental threat (Davis *et al.,* 2002) or impair the gut function (Cromwell, 2001; Davis *et al.,* 2002). MOS can be used in the place of Cu in pig diets to reduced these problems (Davis *et al.*, 2002). MOS has the ability to attach to mannose binding proteins on the cell surface of some strains of bacteria, thereby preventing these bacteria from colonising the intestinal tract by interfering with the binding of carbohydrate residues on epithelial cell surfaces (Davis *et al.*, 2000).

1.1.14 Other claims and examples of probiotics

The digestive tract is most frequently the objective of the functional and health claims and a large market already exists for the gut functional foods worldwide (Lalles *et al.*, 2004; Cummings *et al.*, 2004). The most widely used probiotics are lactobacilli and bifidobacteria that can survive in the intestines. Numerous investigations have been conducted on the beneficial effects on human health for these species (Perdigon *et al.*, 1990; Benno *et al.*, 1996; Shek, 1976, (cited for all in Yu *et al.*, 2004)). Reports of a culture of *Lactobacillus acidophilus* actively taking up cholesterol from laboratory media have been validated, and it is known to beneficially modify serum cholesterol levels (De Rodas *et al.*, 1996 (cited in Yu *et al.*, 2004 and in Adjiri-Awere & van Lunen, 2005)).

In addition, *Lactobacillus* and *Bifidobacterium* sp. have been found to be involved in functions that include inhibiting disease causing bacteria, antitumour and anticholesterolaemic activity, positive effects on digestion, and exhilaration of immune system (Piard and Desmazeud, 1991; Adachi, 1992; Wu *et al.,* 2001; Lin *et al.*, 2002; Yu *et al*, 2004; Adjiri-Awere & van Lunen, 2005). Supplementation of

lactic acid bacteria (LAB) to piglets as been discovered to promote body weight gain, improve feed conversion, boost the populations of bacteria, and lower levels of pathogenic intestinal bacteria. In other studies conducted, it was found that a *Lactobacillus acidophilus, L. pentose* and *Bacillus subtilis* mixture could regulate intestinal microbes, boost immune response and lower serum cholesterol (Lin *et al.*, 2002). LAB do not necessarily need to adhere to or colonise the gastrointestinal tract, as long as they are regularly consumed. Many species and strains of LAB from several genera have been credited with these health benefits, due to their ability to produce different types of antibacterial compounds. They are consumed through many different commercially available products (Lin, 2000). Generally a product, depending on the type, can contain one or more of the following species: *Streptococcus thermophilus, Lactococcus lactis, Leuconostoc mesenteroides, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus acidophilus*, and *Lactobacillus reuteri* and some *Bifidobacterium species*. In addition, products can contain other *Lactobacillus species*, although some are even currently not regarded as a species (such as *Lactobacillus caucacicus* in some products sold in health feed stores) (Lin, 2000).

There is proof that some probiotic bacteria can arrest cell attachment and cell invasion by enterovirulent bacteria. Lactobacilli and bifidobacteria are easily cultured and have a good safety record (Lin, 2000). On the other hand, other studies have not identified any improvement pig in growth performance (Harper *et al.*, 1983; Yu *et al.*, 2004), and this is in agreement with Stewart and Chesson, 1993. Some investigators still insist that probiotics cannot replace antibiotics (Walton, 2001; Partridge, 1991 (cited in Stewart & Chesson, 1993)). Others think that probiotics are more effective in young animals because: 1. nutrients are more efficiently absorbed because of the thinner small intestinal epithelium; 2. nutrients are spared due to a reduction in competing microorganisms; 3. microorganisms responsible for subclinical infections are reduced or eliminated; 4. production of growth-depressing toxins or metabolites by the gastrointestinal microbiota is reduced; 5. microbial deconjugation of bile salts is reduced (Jensen, 1998). Antibiotics should continue to be used in older animals where probiotics are thought to be less effective and antibiotics should continue to be used when a danger of disease is sensed (Walton, 2001).

In other studies, it has been concluded that spores of *Bacillus subtilis* germinate in the digestive tract (Casula & Cutting, 2002; Link *et al.*, 2005). Because the vegetative forms are easily affected by bile salts one may suspect their consequent sporulation or lysis. It is most likely that the spores themselves apply a probiotic effect by acting as stimulators and increasing local cell-mediated immunity (Caruso *et al.*, 1993 (cited in Link *et al.*, 2005)). However in the study by Link *et al.*, 2005, they did not observe significant differences in either phagocytic activity or polyclonal activation of lymphocytes isolated from the peripheral blood of pigs. In the experiment by Apgar *et al.* (1993), *Streptococcus faecium* treatment of weaned pigs had no effect on cell-mediated immune response and Apgar cited other similar results in the study by Kluber *et al.*, (1985). Other investigators observed an increase in macrophage activation in mice fed *Lactobaccilus casei* and *L. bulgaricus*. Mice treated with *S. thermophilus* have also shown to have higher phagocytic activity of peritoneal macrophages and the reticuloendothelial system (Perdigon *et al.*, 1987; Apgar *et al.*, 1993). Similar research from this station (Clayton, 2002) reported that in addition to vitamin C, the main stimulator of non-heam iron absorption in the diet is meat (Seth & Mahoney, 2000 (cited in Clayton, 2002)). During digestion, gastric acids denature the proteins before they are split into smaller particles as peptides and free amino acids (Totara, 1996 (cited in Clayton, 2002)). Kroe *et al.* (1963) (cited by Clayton (2002)) reported that nine essential amino acids found in meat were capable of promoting the uptake of ferrous iron (and histidine, glutamine, glutamic acid and methionine), whereas cystein, histidine and lysine were able to increase ferric iron uptake. Cysteine, comparable to vitamin C is a reducing agent and helps to transform ferric iron into ferrous form.

1.1.15 Some requirements for development of probiotics

The understanding of the structure and operation of the gastrointestinal tract microbial communities and into the activity of the specific microbial species within this ecosystem is important for the development of rational alternatives to in-feed antibiotics, such as probiotics and prebiotics. The wide variety of bacteria in the gut give it a unique environment. The background knowledge on digestion, together with the wide variety of foods offered for consumption today make for great diversity in

individual patterns of digestive and immune function (Cummings *et al.*, 2004; Scheinbach, 1998; Stavric, 1992).

Many experiments have been conducted to distinguish microbes in faecal samples. It has been possible to spot gram negative and gram positive bacteria while strict anaerobes have been difficult to identify using conventional means. The new development of ribosomal RNA (rRNA) as a molecular marker, backed by expertise in animal nutrition, and knowledge of elements that dictate the composition of the microbiota and the invasion of pathogenic organisms in the GIT, will lead to a better understanding and identification of factors concerning the gut microbiota that affects the ecosystem of the gut at different times, and how these can be manipulated to maintain a normal, working and healthy gut (Lalles *et al.*, 2004).

Many beneficial claims have been made and at this point it is crucial to set the boundary or standards between normal and ill health, the benefits to the host's health and how to measure aspects of digestion and immunity and interpret the results (Cummings *et al.*, 2004).

1.1.16 Cost and effectiveness of probiotics

Although natural products like enzymes from bacteria have been known to improve performance, the method of production can be expensive with low tolerance to heat (Lopez, 2000). Improvement of production methods will increase effectiveness, lower their price and increase the amounts to be used (Lopez, 2000; Scheinbach, 1998). It has been observed that some probiotics can be used in low quantities comparable to antibiotics with similar effects (Lopez, 2000).

1.1.17 Interactions between probiotics, ingredients and time

As Fugh-Berman (2000) and Chavez *et al.* (2006) reported herb-drug interactions or, in our our case, probiotic-dietary ingredient interactions are evident and probiotics researchers should advise users about mixing probitics and diet formulations and timing of their use. They need to know whether side effects would arise from using

these preparations in certain circumstances, such as in combination with drugs or other ingredients (Fugh-Berman (2000) and Chavez *et al.* (2006). For example, although there is proof of recombinant human lactoferrin (rh-LF) reducing bacteraemia and lowering disease severity scores in infants, oral treatment with rh-LF failed to protect patients with *E. coli* (Edde *et al.*, 2001).

1.2 The immune system

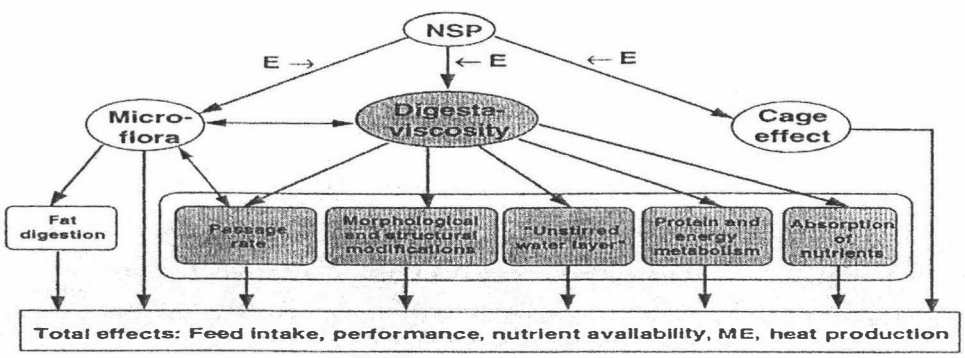
Since probiotics are said to stimulate the immune system, it is relevant to know how the immune system operates. The variety of claims for gut health and immune function are found on food on the European market. Generic claims such as 'help keep your body in balance with the probiotics' and 'diets rich in fibre keep your digestive system regular' are common. Further benefits associated with intake of probiotics are widely reported in magazines. The claims made can be broadly categorized into content, functional, enhancement function, reduction of disease risk or disease risk factor and medical claims (Cummings *et al.*, 2004).

1.3 Effect of natural products on pig performance

1.3.1 Effects and mode of action of non-starch polysaccharide (NSP)

Various reviews have been made by numerous researchers such as: Pearson and Dutson (1991), Cumming *et al.* (2004), Lalles *et al.* (2004), Lopez (2000), Atherton and Rubbins (1987), Jensen *et al.* (1998), Jensen & Jensen (1998), to mention but a few.

Figure 1: Assummed mode of action of nonstarch polysaccharides (NSP) and NSP-hydrolysing enzyme (E)



From Simon, 1998 p116

Growth of indigenous microbiota identified as having beneficial properties on the host, may also be added to the gut. This may be done by the incorporation of compounds in the diet which survive passage through the stomach and small intestine and selectively stimulate selected bacteria in the hind gut. Carbohydrates such as non-starch polysaccharides (NSP), are the principle energy substrate for large intestinal fermentation in pigs (Jensen et al., 1998). NSP and oligosaccharides may pass undegraded to the colon and may influence the composition of microbiota in the large intestines and thus directly affect the total effects (e.g. increase performance by improving feed intake or through decreasing the concentration of ammonia in the hind gut: cage effect (Jensen & Jensen, 1998).

In addition to cage effect, NSP can also stimulate enzymes to improve microflora and reduce digesta viscosity. Microflora can affect either digesta viscosity, or total effects through improved fat digestion or can directly influence these effects. When digestion viscosity is improved it then stimulates intermediate factors which all or separately improve some total end effects (Simon, 1998).

1.3.2 Effect of probiotic on blood parameters

Intepretation of leucocyte concentrations in the blood provides insight regarding potential processes that may be occurring in the patient. The leukogram is complete set of numerical data in the leucocyte profile, along with any noted morphological abnormalities. Abnormalities in the leukogram might provide inferences to a pathological process such as inflammation but might not necessarily establish a specific diagnosis. The correct interpretation of leukocyte abnormalities and together with clinical findings, however, may lead to a diagnosis (Thrall *et al.*, 2004; Tvedten, 1993; Voigt, 2000; Sims, 1996). Blood examination can be used to detect health status and for production monitoring. It can also be used for early detection of disease and blood disorders (Evans, 1994).

Exellent review in the field of interpretation of blood parameters can be obtained from blood interpretation manuals from (electronic) counting machines such as the ADVIA 120 and by reaseachers such as Egeli *et al.*, 1998 and Thrall *et al.*, 2004).

1.3.3 Effect and mode of action of Lactoferrin on performnance and general health of pigs

The mucosa of the gut is the first line of contact with pathogenic microorganisms that are ingested by the animal and has essential functional role of functional barrier against these agents (Bosi, 2000). Non-immunological and immunological components of the gut mucosal barrier are depicted in Table 1.

Among biologically active proteins and peptides in milk are glycoproteins and lactoferrins. Proteins and peptides in glycoproteins are lactoferrin, milk mucins (e.g. mannose containing glycoproteins), and adhesion molecules, while those in lactoferrin are lactoferroxins (Zabielski, 1998). To understand the effect of lactoferrin on animal performance, we have studied the reviews on the chemical and biological properties by Cumberbatch *et al.* (2000); De Wit & Hooydonk (1996); Reiter (1985), Reiter & Perraudin (1991), Iyer & Lonnerdal (1993), Lonnerdal & Iyer (1995), Wong *et al.*(1997); Lonnerdal (2003), to name but afew. The structure and functions of lactoferrin, as reviewd by the following researchers such as Lonnerdal & Iyer (1995); Tome & Debbabi (1998); Muri *et al.* (2005) have also been studied. Lactoferrin is a bioactive protein which makes an important contribution to the host defence system. It eliminates pathogens such as bacteria, viruses and fungi, stimulates and protects cells involved in the host defence mechanisms and controls the cytokine response (Steijns, 2001; Prgomet *et al.*, 2005). Present commercial bovine lactoferrin products are included in infant formulas, nutritional iron supplements and drinks, fermented milks, chewing gums, immune-enhancing nutraceuticals, cosmetic formulas and pet care supplements (Steijns, 2001).

Bovine lactoferrin is used in cats for the treatment of intractable stomatitis by stimulating the phagocytic activity of neutrophils (Steijns, 2001). It also induces both mucosal and systematic immune response in mice (Steijns, 2001).

Monocytes, neutrophils and macrophages are cells of the immune system that kill invading pathogens by oxidant reactions. As free iron is often present in the areas of inflammation or infection, these oxidant reactions may be accelerated due to the catalystic effect of iron on free radical production. Lactoferrin will bind the free ferric

iron with high affinity, and thus function as a local antioxidant, protecting the immune cells against the free radicals produced by themselves. Although only the neutrophils degranulate and deliver lactoferrin, monocytes and macrophages have lactoferrin receptors on their cell surface (Tome & Debbabi, 1998).

Protein components of milk have many uses. They provide amino acids which are required for growth and development. They also have more specific functions. The nature of the peptides formed during milk proteins digestion is dependent on the digestive process. The biological properties of milk proteins or milk protein-derived peptides include protein with antimicrobial activity (lactoferrin), peptides which promote nutrient assimilation and peptides with modulatory activity on the physiological function (Tome & Debbabi, 1998).

The role of the different pathways in hypothetical functions, i.e. immune surveillance, metabolic regulation or gastrointestinal disease, still remains unknown. This absorption of large molecules in antigenic and biologically active quantities are believed in particular to play a role in different physiological and immumunological responses that contribute to humoral tolerance and regulation (Tome & Debbabi, 1998).

Different proteins including lactoferrin, vitamin B_{12} s binding protein, folate binding protein, β-lactoglobulin and α- lactalbumin, are assumed to interact with either minerals, vitamins or nutrients by a specific mechanism. These interactions may have an effect on absorption of these nutrients (Hanson *et al*., 1994; Tome & Debbabi, 1998).

Lactoferrin has been particularly studied for its role as an iron-scavenging protein that could be involved in iron transport, or have an anti-bacterial, anti-inflammatory and immunomodulating properties (Iyer & Lonnerdal, 1993; Tome & Debbabi, 1998).

1.3.4 Common methods of preparation / production of lactoferrin

Lactoferrin and glycoproteins are among biologically active proteins and peptides in milk and they are claimed to have beneficial effect on the host (Zabielski, 1998). Bovine lactoferrin may be isolated from fresh skim milk by cation exchange chromatography and gel filtration. Milk at native pH is passed for a short time through S Sepharose fast flow at 4 C and the attached proteins eluted in steps with 0.1, 0.35, and 1 M Nacl, respectively. The 1 M NaCl fraction containing lactoferrin is then dialysed and freeze dried. The resulting material is then dissolved in 25 mM sodium phosphate buffer at pH of 6.5 and reapplied to the cation exchanger, which is earlier equilibrated in the obove buffer. Lactoferrin is then eluted by application of salt gradient to 1 M NaCl in phosphate buffer and the recovered material dialysed and freeze-dried. Final purification of lactoferrin is achieved by gel filtration through Sephacryl S300 in phosphate buffer and the protein recoverd as a dialysed freeze-dried powder. Purity of the final product is usually greater than 98% as assessed by resource reversed-phase high performace liquid chromatography (HPLC) and mono-SHPLC (Palmano & Elgar, 2002, cited in Cornish *et al.*, 2004)).

1.3.5 The normal or reference values of the leukogram

Before making a conclusion concering what is normal or deviates from normal, a number of steps should be followed in order to intepret the leukograms. Intepretative attention should concentrate only on the absolute values within the differential count. When examining the haematology report, the total leucocyte concentration should be the first thing to look at. The total leukocyte count is not directly interpreted but is only used to calculate absolute differential concentration. If the total count is decreased, the absolute concentration of each type of each type should be examined to determine which are deficient. If the total count is decreased, examine the absolute concentration of each cell type to determine which are present in excess. Even if the total concentration is normal, examine the absolute concentration of each cell type if any abnormalities in distribution are present. Identified abnormalities in the absolute concentration of individual leukocyte types are then interpreted into processes. (Thompson & Forsyth, 2004; Tizard, 2000; Thrall *et al.*, 2004; Voigt, 2000 and Egeli *et al.*, 1998; Comazzi *et al.*, 2004).

The electronic hematological cell counter is able to provide a differential white cell count, isolating the number of white cells in each of the blood samples as shown in Table 2. The acceptable physiological values of the red blood cells parameters in a normal health pig are shown in Table 3.

Table 2: Blood parameters, their abbreviations and units of measurement

White cells	WBC	x 10^9cells/L
Neutrophil cells	NEUT	x 10^9cells/L
Lymphocyte cells	LYMPH	x 10^9cells/L
Monocyte cells	MONO	x 10^9cells/L
Eosinophil cells	EOS	x 10^9cells/L
Basophil cells	BASO	x 10^9cells/L
Red blood cells	RBC	x 10^{12} cells/L
Haemoglobin concentration	HGB	g/L
Haematocrit level	HCT	L/L
Mean cell volume (or mean erythrocyte volume)	MCV	fL
Mean cell haemoglobin (or mean erytrocyte haemoglobin content)	MCH	p g
Mean corpuscular haemoglobin concentration (or mean erythrocyte haemoglobin concentration)	MCHC	g/L
Corpuscular haemoglobin constant	CH	pg
Corpuscular haemoglobin concentration mean	CHCM	
Corpuscular haemoglobin	CH	pg
Red blood cell distribution width (or erythrocyte distribution width)	RDW	%
Haemoglobin distribution width	HDW	g/L
Platelet	PLT	x 10^9 cells/L
Mean packed volume	MPV	fL
Packed cell volume	PCV	%

Table 2: Accepted physiological values of white and red cell parameters in the normal and anaemic pigs
(Adapted from Egeli et al. (1998), Clayton (2002), Miller et al. (1961), Tumbleson & Kalish (1971), Svoboda et al. (2004), Ullrey, 1959)

Blood parameters	Abbreviation	unit of measure	(Egeli) range	percent of white cell mass	(Miller) et al. 1961)	(Tumbleson & Kalish, 1971)	(Advia 120)	(Svoboda et al., 2004)	Ullrey et al., 1959	Ruakura
			Age (days) (x-x)		Age (21-49) normal	Age (42) normal	Age (X-x)	Age (7-35) lowest	Age (24-31) anemic- healthy	Age (x-x)
White cells	WBC	x 10^9cells/L	10-23	100	x	x	x	x	6.92-9.34	5.0-8.0
wbc type										
Neutrophil cells	NEUT	x 10^9cells/L	2.5-10	26.6-56.7	x	x	x	x	13-1.9	
Lymphocyte cells	LYMPH	x 10^9cells/L	7.0-15.5	35.5-62.0	x	x	x	x	8.1-7.3	4.5-13.0
Monocyte cells	MONO	x 10^9cells/L	0.32-2.0	1.6-8.8	x	x	x	x	0.2-0.7	0.2-2.0
x										
Eosinophil cells	EOS	x 10^9cells/L	0.08-1.76	0.1-5.6	x	x	x	x	0.2-0.2	0.5-2.0
Basophil cells	BASO	x 10^9cells/L	0-0.3	0-2.7	0.0	x	x	x	0.0 -0.2	x
				Method of calculation						
Red blood cells	RBC	x 10^{12} cells/L	5-8x10^{12}	direct	x	3.16-5.65	x	4.5	2.29-5.0	5.0-8.0
red cell type and parameters										
Haemoglobin concentration	HGB	g/L	110-170	direct	90±0 2	82-108	x	80	40- 98	100-160
Haematocrit level	HCT	L/L	0.37	direct	0.304±0.08	0.26-0.36	x	0.3	0.12-0.2	0.32-0.50
Mean cell volume	MCV	fL	50-68	x	x	54.6-97.5	x	55	44.2-55.6	50-68
(or mean erythrocyte volume)										
Mean cell haemoglobin	MCH	p g	14.4-20.1	HGB/RBC	x	16.2-29.4	x	17	x	17-21
(or mean erytrocyte haemoglobin content)										
Mean corpuscular haemoglobin concentration	MCHC	g/L		calculated as:						
(or mean erythrocyte haemoglobin concentration)			300-340	HGB/RBC x Mean	280.5±2	275-320	x	280	310-340	300-340
Corpuscular haemoglobin constant	CH	g/L	x	MCHC or MCH/MCV	x	x	x	x	x	x
Corpuscular haemoglobin concentration mean	CHCM									
Red blood cell distribution width	RDW	%	x	x	x	x	x	<19.9	x	x
Plama iron concentration		µmol/l	x	x	x	x	x	10	x	x

Egeli, 1998	crossbred (Hampshire x Duroc)	Ullrey, 1959:	-
Miller et al., 1961	-	Tumbleson & Kalish (1971)	crossbred (Hampshire x Duroc)
Svoboda et al., 2004	-	Ruakura Animal Health Laboratories, Hamilton	-
Advia manual	-		

1.3.6 Interpreting the results

Many researchers have tried to define the boundaries or standards between normal and ill health, the benefits to the host health, how to measure aspects of digestion and immunity and interprete the results (Cummings *et al.*, 2004).

Measuring aspects of immune function is possible, but there is no one test that will define either the status of functional capacity of the immune system. Human studies are often limited to the ability to sample secretions such as blood and saliva but it should be remembered that only 2% of lymphocytes circulate at any given time, which limits intepretation of data (Cummings *et al.*, 2004).

Knowledge of normal leukocyte (Table 2), erythrocytes and erythrocyte indices is important as this can lead to identification of movements away from normal to disease status (Thrall *et al.*, 2004; Voigt, 2000).
Increases in cell numbers, in particular cell type, is suffixed as –philia or -cytosis such as –basophilia -increase in basophils, whereas a decrease in particular cell types are suffixed with –penia for example decrease in neutrophil is called neutropenia (Thrall *et al.*, 2004).

Studies in computer derived haematological graphics can help a clinician to diagonize abnormalities in the animal due to stress or ill health.

1.3.6.1 Evaluation of leukocytes

In normal or healthy animals, leukocyte morphology and numbers are relatively static, but changes often occur in illness. Such changes are seldom specific but none provide conclusive information concerning the nature of the disease, and the prognosis. Leukocytes are routinely analysed by estimation of the total and differential leucocyte counts (Thompson & Forsyth, 2006).

Leukocytosis (increased WBC count) is most usually fueled by a neutrophilia rather than by eosinophilia, lymphocytosis or monocytosis. Similarly, leucopenia is in most cases caused by a neutropenia (Thompson & Forsyth, 2006).

1.3.6.2 Differential leucocyte count

These are obtained by examination of stained blood smear samples until 100 or 200 leukocytes have been identified and classified. Leukocytes are usually reported as a percentage as well as the absolute number of each cell type (% x WBC). Interpretation of the differential count is based on the absolute numbers of each cell type although percent may be used (Thompson & Forsyth, 2006).

Leukocytes are used in body defense mechanisms and can encounter a wide variety of different infectious agents or foreign material. This is made possible through phagocytosis and antibody production. Leukocytes with phagocytic properties include granulocytes (neutrophils, eosinophils and basophils) and monocytes. Lymphocytes are not phagocytic, but are take a protective role in antibody production and cell-mediated immunity. Despite these different functions, the two systems often work together in defending the body. For example, macrophages are required to "process" antigen for the B-lymphocytes, which then produce specific antibodies. Bacteria coated with antibodies produced by lymphocytes can be more efficiently phagocytosed by neutrophils (Thompson & Forsyth).

In this literature review, it can be summarized that probiotics have many effects on the host. It is now therefore important to find better aids such as ribosomal RNA (rRNA) (Lalles *et al.*, 2004) and conduct statistical and significant researches to set the boundary or standards between normal and ill health, the benefits to the host's health and how to measure aspects of digestion and immunity and interpret the results (Cummings *et al.*, 2004).

Chapter 2.

2. EFFECT OF PROBIOTIC- AND LACTOFERRIN-SUPPLEMENTED DIETS ON GROWTH PERFORMANCE, BLOOD PARAMETERS AND FAECAL SCORES IN WEANLING PIGS

2.1 Introduction

Probiotics and other natural substances should be tried following a ban on antibiotic in the animal feed industry. Researchers in the animal and feed industry are now trying some alternatives and natural products that range from chemicals and organic acids to biological products such as probiotics. Probiotics produce variable and unpredictable effects and their mode of action is not fully understood (Jensen, 1998).

The aim of this experiment is to look at the effects of five diets different diets (a control or basal diet verses three probiotic- and lactoferrin supplemented diets) on average daily gain, ADG, average daily feed intake, ADFI, feed concersion ratio, FCR, body weight, immunity, MWFS (scouring), stress factors (L/N ratio) and general health performance in weanling pigs. Other studies may have looked at different probiotics, environment, species, concentration etc but we think that not many people have look at using lactofferin (diet E) in piglets diets. The other three probiotics (B, C and D) used in this study are not known (names withheld by the sponsor) except for lactofferin and therefore a wide literature search has been made in order to match the effects of these natural products with the ones used in the current study.

2.2 Materials and Methods

2.2.1 Experimental Animals

One hundred and sixty weanling pigs (four weeks old, average body weight 7.453 kg) of different sexes were obtained from four different farms and used in a 21-day experiment. There were two identical trials with eighty piglets each conducted at two different times. Of the four farms, three had Large White x Duroc cross breeds while

the forth had Camborough breed. Upon arrival, the piglets from the four different farms were mixed to place them in an immune challenging situation.

The first trial was conducted from 14 November to 5 December 2005 and the second from 14 February to 26 March 2006 using a factorial arrangement. In each trial the eighty piglets were identified by ear tags and randomly allotted within litter to:
[a] five treatments as follows: 1.diet A (with no added probiotic) control group, 2. diet B group (control added with probiotic B), 3. diet C (control added with probiotic C) group, 4. diet D group (control added with probiotic D) and 5. diet E (control added with lactoferrin) group;
[b] five different rooms (climatically controlled with standard indoor conditions) and were blocked by weight. Within each run (of eighty piglets each) there were four litters with five piglets each from each farm.

The following data was recorded:
1. individual body weights on days 0, 7, 14, 21. 2. pen feed intake over 7-day intervals, 3. pen weekly feed conversion, 4. weekly average gain, 5. daily diarrhoeal/fecal scores, 6. daily room temperature and 7. hematological data from blood samples collected on day 0, 14 and 21. ADG, ADFI and ADFCR at the end of the experiment was calculated using body weight at start of the experiment as a covariate.
Health status was evaluated daily by visual appraisal on the following clinical traits

Collected data was analysed to evaluate the effect of diet, farm, sex and room on the piglets. Two piglets were housed per pen and sixteen pens per treatment.

During the experiment, pigs were managed in accordance with requirements of animal protection approved by the Massey University Animal Ethics Committee (Ethics application number MUAEC 05/85).

2.2.2 Weaner pens

The site of the experiment was at Massey University Pig Biology Unit, Reproductive and weaner facility, Palmerston North, New Zealand.

There were five rooms, each with 8 pens. Rooms measured LxBxH: 3.69m x 4.76m x 2.2 m. Weaner pens measured L x B x H: 2.20 x 0.64 x 0.65 m. The floor area of each pen was 2.20x 0.64m with 0.90 m x 0.64 closed wooden floor (resting and feeding area) and 1.3 m x 0.64 m plastic-coated metal slat area, with drinking water on this area. The slats were 5.5cm wide and 2cm apart. Urine and faeces could pass through and drop into the drain below the slatted floor. The rooms were totally enclosed and climatically controlled (mechanically ventilated and heated) to maintain pigs in their thermo-comfort zone at $29^{o} \pm 1.5^{o}C$. Temperature was measured and recorded at all times using temperature loggers. Faeces, urine and contaminated feed on the wooden floors were removed regularly.

2.3 Experimental diets and Feed management

2.3.1 Experimental diets

The experiment was conducted over 21 days. The ingredients and the calculated nutrient composition of the basal diet are presented in Table 4.

The experimental diets were designed as:

TRT1: The barley-wheat-soybean meal diet without probiotic (the basal diet or diet A or control diet)

TRT2: The basal diet supplemented with probiotic B (diet B)

TRT3: The basal diet supplemented with probiotic C (diet C)

TRT4: The basal diet supplemented with probiotic D (diet D)

TRT5: The basal diet supplemented with lactoferrin (diet E)

All diets were offered in pellet form. Piglets were given ad libitum access to feed (in movable plastic feeders) and water (from automatic nipples) throughout the experiment.

The percentage ingredients and nutritive composition (calculated nutritive values) of all the diets are shown in Table 4. All diets were formulated to meet or exceed NRC (1998) requirements for all nutrients regardless of treatment and were balanced in energy, amino acids, mineral and vitamins. Except for the addition of probiotics or lactoferrin, all the diets were equal concerning metabolic energy, digestible crude protein, essential amino acids, minerals and vitamins. The diets contained: 21% crude protein, 14.82 MJ/kg digestible energy, 0.95% calcium, 0.75% phosphorus, 1.05% lysine and 11.65% A.D. lysine.

Table 4: Ingredients and diet formulation of experimental diets used in the study to find the effect of different probiotics on performance of weaned piglets

Items	Treatments (TRT)[a]				
	A	B	C	D	E
Ingredients, %					
barley	*25*	*25*	*25*	*25*	*25*
wheat	44.94	44.94	44.94	44.94	44.94
soybean	5	5	5	5	5
fish meal	6	6	6	6	6
skim milk powder	12	12	12	12	12
soy bean oil	3	3	3	3	3
lysine	0.3	0.3	0.3	0.3	0.3
methionine	0.17	0.17	0.17	0.17	0.17
threonine	0.19	0.19	0.19	0.19	0.19
dicalcium phosphate	3	3	3	3	3
sodium chloride	0.1	0.1	0.1	0.1	0.1
vitamin-mineral premix[b]	0.3	0.3	0.3	0.3	0.3
probiotic[c]	-	B	C	D	E
Nutrient composition (calculated values)					
digestible energy(MJ/kg)	14.82	14.82	14.82	14.82	14.82
crude protein, %	21	21	21	21	21
lysine, %	1.05	1.05	1.05	1..05	1.05
Ca, %	0.95	0.95	0.95	0.95	0.95
P,%	0.75	0.75	0.75	0.75	0.75
A.D Lysine	11.65	11.65	11.65	11.65	11.65

[a] Diet A (control), B, C, D and E (lactoferrin)

[b] Each 3.0 kg pack of vitamin and mineral premix (trade name Vitastart, supplied by Vitec Nutrition Limited, Auckland) contained (A) vitamins, MIU:vit A 15.0; vit D 2.0; g: vit E 70; vit K 2.5; vit B1 2.0; Vit B2 3.0; vit B6 2.0; vit B12 0.03; calcium pantothenate 20.0; niacin 20.0; biotin 0.1; folic acid 0.5; choline 150.0; and (B) trace minerals, g:Fe 100.0; Mn 45;Co 0.5; Se 0.3; Zn 120; Cu 125; I 1

[c] Except for diet E, lactoferrin, actual names of probiotic B, C and D, the amount added, concentration and viability were withheld by Fonterra Ltd.

2.3.2 Feed management

The pigs were supplied with sufficient feed. Weekly feed intake prediction equations were generated using step-wise regression procedures. After formulating the diets for the five specific groups, the nutrient requirements of the individual pens were met by weekly adjustment on the basis of the expected BW of that pen for the coming week. The projected BW was estimated by the following formula:

$BW_{estimate, kg} = BW_{today} + 3.5 \times ADG_{previous\ week}$

The regression equations were then used with the BW estimate to calculate a new daily feed allowance for the pen. The feed intake was adjusted after each weighing and the formula was: g/d per pig = 44.6 + 48 x liveweight. Average daily feed intake, feed conversion efficiency and daily gain was calculated weekly for weekly data. Feed was given ad libitum from four-hole self-feeders and consumption was monitored daily. (Feed left over at beginning of week + feed added during week = Total feed offered; Feed eaten = Total offered – feed at end of week). One drinker per pen located over the slatted floor area served as the water source.

2.4 Source and maintenance of cultures

Aqueous forms of probiotics (B, C, D and E [lactoferrin], Fonterra Laboratories Ltd, Palmerston North, New Zealand) were used in these investigations. 20ml of each probiotic was diluted with 1980ml water to make 1,000 ml mixture. This increased volume facilitated the application and mixing in 100 kg pelleted feed. The mixing was carried out slowly by atomization and mechanical homogenization using an electrical horizontal mixer over 25 minute period.

The basal feed (diet A) (control) was made at the Feed Processing Unit of Massey University. The feed was stored in a cool dry place and was consumed within seven days after mixing.

2.5 Colony forming units, CFU

The organisms' viability and concentration (colony forming units, CFU) was not checked during this experiment. Mixing of cultures and the addition of culture to feed was done according to Fonterra's (the manufacturer's) instructions/guidelines.

2.6 Faecal scoring

Subjective scour scores were determined by visual appraisal of each pen on daily basis. Fluidity was scored on a seven–point scale, where:

1 = hard, dry and cloddy (rarely seen); 2 = firm; 3 = soft but able to retain some shape (no sours); 4 = soft and unable to return any shape; 5 = watery and dark; 6 = watery and yellow, and 7 = foamy and yellow (severe scours).

This was done every morning, by checking weather hard or watery and giving them a score. Faeces from each piglet (pen) were collected on days 0, 7, 14 and 21 and stored under frozen refrigeration.

The survival rate and mean weekly faecal scores were calculated. Mean weekly faecal scores (MWFS) were determined by totaling the highest scores for each day for 7 days and dividing by 7. MWFS were determined by totaling the highest scores for the days recorded in that week and dividing by the number of entries in the week.

2.7 Blood Sampling

Blood samples for the determination of selected parameters for haematological examination were collected on day 1 (arrival), 14 and 21 of the trial from each piglet. Blood was sampled from the anterior vena cava using a 20 Gauge 1 or 1.5 inch needle and collected in 5 ml vacutainers (BD Vacutainer^{TM}; Becton, Dickinson and Company, NJ, USA) with anticoagulant ethylene-diamine-tetra-acetic acid (EDTA). Two 5ml blood samples (about 2% of the total blood volume) were taken using the vacutainer collection systems. The piglets were held head downwards.

Immediately after the blood collection from each piglet, the tube with anticoagulant EDTA [0.068 ml of 7.5% (K_3) EDTA solution (5.1 mg)] was agitated to mix blood by inverting manually or using the electrical blood mixer. One set of the blood samples (80 tubes) were then transported to Fonterra Microbiology lab and the other (80) to Massey University, Institute of Veterinary, Animal and Biomedical Sciences (IVABS) laboratory for determination of selected parameters for haematological examination using an automated haematology analyzer, the "Bayer Advia 120" electronic cell counting apparatus (Bayer Corporation, Tarrytown, New York, USA

2.8 Haematological / immune status

Unclotted whole blood samples were used in the laboratory to determine packed cell volume (PCV), total red and white cell counts and differential white cell count as well as the haemoglobin content of the sample. These parameters were then used to assess a number of other characteristics of the red and white blood cell population. Total white blood cell count was performed along with a differential white cell count to facilitate interpretation of the results of the tests.

Blood cells, which include white blood cells (WBC), neutrophil cells (NEUT), lymphocyte cells (LYMPH), monocyte cells (MONO), eosinophil cells (EOS), basophil cells (BASO), red blood cells (RBC), haemoglobin concentration (HGB), and erythrocyte indices viz., haematocrit level (HCT), mean cell volume (or mean erythrocyte volume) (MCV),
mean cell haemoglobin (or mean erythrocyte haemoglobin content) (MCH), mean corpuscular haemoglobin concentration (or mean erythrocyte haemoglobin concentration) (MCHC), corpuscular haemoglobin constant (CH), Corpuscular haemoglobin concentration mean (CHCM), red blood cell distribution width (or erythrocyte distribution width)(RDW), haemoglobin distribution width (HDW), platelet (PLT) and mean packed volume (MPV)and leukocytes viz., neutrophil (NEUT), lymphocyte (LYMPH), monocyte (MONO), eosinophil (EOSI) and basophil (BASO) were determined using automated hematology analyzer, the "Bayer Advia 120" electronic cell counting apparatus (Bayer Corporation, Tarrytown, New York, USA). The 'Bayer Advia 120' automated hematology analyzer analysed the blood samples and presenting it in form of cytograms and histograms.To prevent double handling and to reduce stress, the pigs were weighed just before sampling.

The electronic hematological cell counter was also able to provide a differential white cell count, isolating the number of white cells in each of the blood samples.

2.9 Statistical analysis

Data were analysed using the GLM procedure of SAS (SAS Institute Inc, Cary, North Carolina, USA). Repeated measures analysis was used as data were collected over time. The statistical model for growth parameters included weight of pigs at the beginning of the study, diet, replica, farm, sex and interaction of farm x diet. The five diets were evaluated to find their effect on growth performance, health and haematological status on weaner pigs. For animal performance, pen was considered as the experimental unit. The preliminary model for average daily gain, feed intake and feed conversion rate included diet, weight block, replica, farm, sex ant the interaction farm x diet. Initial weight was used as a covariate.

For blood parameters, fecal score and stress factors, individual animals served as the experimental unit. Each variable was correlated with a every other variable to identify possible relationships.

Analysis of variance (ANOVA) was used to compare the mean initial weight of the five diet groups. The results are presented as least square means (LSM) with associated standard error (SE). Differences were considered significant at $p < 0.05$, although P values up to $\leq .0.10$ are shown in the text if the data suggest a trend. When a significant F-test was found, means were separated using the least significant difference test.

Measures of physical performance that is ADG, AFI and AFCR, hematological/immune status were analysed using the mixed model procedure of SAS (SAS Institute Inc, Cary, North Carolina, USA).

using repeated measures analysis and body weight at the beginning of the experiment was used as a covariates in the statistical model for physical performance. The statistical model was:

$$Y_{ijk} = \mu + D_i + P_j D_i + W_k + D_i W_k + e_{ijk}$$

Where:

Y_{ijk} -is an observation in the k^{th} week of the j^{th} piglet with the i^{th} diet treatment;

μ -is the general mean;

D_i -is the fixed effect of the i^{th} diet treatment;

P_jD_i -is the random effect of the j^{th} piglet within the i^{th}diet treatment;

W_k -is the fixed effect of the k^{th} day time

D_iW_k -is the interaction between the i^{th} diet treatment and the k^{th}day

e_{ijk} -is a random (residual) error unique to Y_{ijk} assumed to be normally and independently distributed with meanθ and variance $\delta^2 r$

CHAPTER 3

3. RESULTS

The data were unbalanced because in week 3 of the study period, two female piglets from two different rooms on diets B and D coming from the same farm (Matton) in run 2 were culled due to Rotavirus and septic polyarthritis respectively. Based on daily animal health management checks, all the trial pigs appeared to be free of major diseases although individually sporadic cases of wasting disease was noted with reduced weight gain, a more prominent spine skinnny pig in one case, skin disease in one pig, inflammations, pneumonia in two, enteritis in three, coughing and diarrhoea (serious and treated in seven), emaciation in one reduced intake, ADG and FCR were detected. Scouring was recorded during the study period but did not appear to be linked with any one treatment group. The immune challenge was evident as pigs in all diet groups had lower weights compared to the standard growth performance and were lower on day 7 and 14 postweaning / immune challenge.

The health status of early weaned piglets was evidenced by the number of piglets that finished the test. Of the initial 160 piglets, 158 finished the test. Rotavirus and *Escherichia coli* are among the major causes of postweaning diarrhoea in young pigs and weaning stresses would possibly enhance the pathogenesis of this agent in the gastrointestinal tract. This was also noted in our study as pigs feed diet B had numerically higher diarrhea scores (high MWFS) which increased in magnitude from day 7 to 14, one piglet was diagnosed of this disease and euthanased on day 20 of the experiment. Although there were two cases of polyarthritis and rotavirus, it was of low magnitude and the other animals appeared to resist the disease as no further cases were observed. No signs of toxicity were observed for any treatment. The pigs with very low feed intake and showing acute case of diarrhoea were given electrolytes [Vitastart (Vitec Nutrition Limited, Auckland, New Zealand). Some pigs were urinating and /or defecating in or near feeders and this may have reduced intake, although weight of left over feed or spilt feed was estimated and fresh feed replenished. Some piglets were jumping from one pen into another leaving some pens with one and the others in three pigs per pen instead of two. At one time one pig

jumped from the pens into the walk area without feed or water and on a cold floor. The pens were of different sizes and made from different materials – wooden boards, metal sheets or welded metal bars and this may have affected results. Differences in number of pigs per pen might have had an effect on the piglet performance (Adjiri-Awere & van Lunen, 2005). Some pigs were able to eat spilt feed from adjacent pens. There was also some physical contact between some pens, pigs licking other pigs on different diets. Self-innoculation was not taken care of.

Manual dilution methods using a haematocytometer gives more error (approximately 20%) and therefore to reduce these errors we used automated haematologic WBC counters which are more accurate (5% error). However, there could have been other errors to the final blood data, due some clumped platelets, perhaps following a difficult and/or prolonged sample collection. Blood collection sometimes became a problem and may be this might have produced some samples of inadequate quality or the results might have been distorted by the effects on animal's haematology of stress or excitement.

Sedation was avoided, since pharmacological intervention can alter the blood results in an unpredictable manner. To minimize degeneration of white cells, that might occur to an extent sufficient to make white cell differentiation unreliable, blood was sent for analyses to nearby laboratory immediately after completion of each sampling. The electronic counter also counts all nucleated cells, including nucleated erythrocytes and there is need, if nucleated erythrocytes (nRBC) are detected in the peripheral blood smear, to correct the number per 100 leukocytes that should be counted and the WBC count corrected by the correction formula.

The location rooms and individual pens in the rooms were also biased because of noise due to opening or leaving doors open during some operations. One day one pen did not have water when they were checked the following morning. The piglets started with lower lymphocyte cell numbers which were below the normal range. The period of immune challenge was too short for the significant differences in immune status to be noticed.

More analysis should be done rather than just visual appraisal of fecal scoring. Some pigs should have been slaughetered and post-mortem conducted as some symptoms can be subclinical.

Due to the variability in white and red blood changes during different stages of growth from one to four weeks post weaning or of age, it was recommended that multivariate analysis, assessing only those parameters relevant to the experimental design, be conducted to adequately evaluate particular disease or nutritional conditions. References values can not be generalised to draw particular conclusions and therefore some values taken may bring about contradicting conclusion s as there are other factors that affect growth performance and blood parameters.

Correlations (R^2) among the criteria responsonses within treatments are presented. Higher correlations were obtained among all data with the exception of weight in week 3 (wk3wt), average daily gain (ADG), average daily feed intake (ADFI), fed conversion ratio, monocytes, eosinophils and mean weekly fecal scores (MWFS) which had lower correlation.

It was noted that on day 0, not all variables were similar. The difference was probably due to random assignment since pigs were randomly allotted to treatment groups.

During the study, trends between the control, probiotic- and lactoferrin supplemented animals were examined to determine whether any differences could be observed in performance and in the blood of the five groups. It is clear that the five diet groups showed different performance and cellular response to that of the animals fed the control diet and this is reported and might be of some use as an indicator of disease or a benefit.

Least-square means of the effects of probiotic-supplemented diets on growth performance in weanling pigs for average pen live weight of piglets at the start of the experiment (wk0wt), average pen live weight of piglets at the end of week 1 (wk1wt), average pen live weight of piglets at the end of week 2 (wk2wt), average pen live weight of piglets at the end of week 3 (wk3wt), average daily gain (ADG), average daily feed intake (ADFI) and average feed conversion rate (FCR) for the 21 day experimental period for each diet with standard error (SE) are presented in Table 5.

3.1 Effect of mixing piglets from different farms

The data showed a strong farm effect, that is mixing of piglets at the start of the trial was significant ($p<0.05$).

3.2 Effect of probiotic- and lactoferrin-supplemented diets on animal performance

3.2.1 Weaning weight (week 0 bodyweight)

BW on day 1 was not different for the five groups (mean values: 7529.8, 7524.3, 7452.5, 7456.5 and 7325.8 g for diet groups A, B, C, D and E, respectively; mean for all group: 7458 g. (Table 5).

Bodyweight at weaning had a significant effect on wk1wt ($p<0.0001$), wk2wt ($p<0.0001$) and wk3wt ($p<0.0001$). Farm had a significant effect on wk2wt ($p<0.05$) and wk3wt ($p<0.05$) while the effect of of farm on wk1wt was not observed ($p<0.05$). However, the effects of diet, replica, sex and the interaction between farm and diet were not significant ($p>0.05$).

Table 5: Least-square means of the effects of probiotic-supplemented diets on growth performance in weanling pigs for average pen live weight of piglets at the start of the experiment (Wk0wt), average pen live weight of piglets at the end of week 1(wk1wt), average pen live weight of piglets at the end of week 2 (wk2wt), average pen live weight of piglets at the end of week 3 (wk3wt), average daily gain (ADG), average daily feed intake (ADFI) and average feed conversion rate (FCR) for the 21 day experimental period for each diet with standard error (SE).

	TRT						
Parameter	A (control)	B (probiotic B)	C (probiotic C)	D (probiotic D)	E (lactoferrin)	SE	p value
Growth Performance							
Weaning wt (g)[1]	7529.8	7524.3	7452.5	7456.5	7325.8	-	-
Wk1wt (g)	8303.05	8423.65	8269.36	8218.81	8010.82	141.97	0.3531
Wk2wt (g)	10441.54	10490.34	10630.63	10223.49	10035.26	249.5	0.4801
Wk3wt (g)	13866.11	14056.9	14000.5	13480.73	13221	362.41	0.4093
Weight gain							
Wk1 – wk0(g)	773.25	899.35	816.86	762.31	685.02		
Wk2 – wk1(g)	2138.45	2066.69	2361.27	2004.68	2024.45		
Wk3 – wk2(g)	3424.91	3566.62	3369.93	3257.24	3185.73		
Wk3-wk0 (g)	6333.3	6532.6	6548	6024.23	5895.3		
ADG (g/d)	305.41	314.49	311.80	287.05	274.69	17.26	0.4093
ADFI (g/pig/d)	404.64[c]	426.77[d]	423.63[cd]	378.48[b]	341.48[a]	19.91	0.0169
FCR	1.34299	1.35963	1.44081	1.35278	1.2574	0.063	0.3776

[1] actual mean values
[a, b, c, d] values in the same row with different letters are significantly different ($p<0.05$).
SE standard error

3.2.2 Week 1 body weight

Bodyweight on day 7 was not significantly different ($p > 0.5$) between the five groups (mean values: 8303.05, 8423.65, 8269.36, 8218.81 and 8010.82 for diet groups A, B, C, D and E, respectively. However, on numerical basis, only the pigs in diet B were heavier than the controls while those in diets C, D and E groups were lighter than the controls. Pigs that consumed diet B were the heaviest while those on diet E were the lightest (Table 5).

3.2.3 Week 2 body weight

Bodyweight on day14 was not significantly different ($p>0.05$) between the five groups (mean values: 10441.54, 10490.34, 10630.63, 10223.49 and 10035.26 for diet groups A, B, C, D and E, respectively. However, pigs in that consumed diets B and C were heavier than the controls while those that received diets D and E were lighter than the controls. Pigs that consumed diet C were now the heaviest while those on diet E were still the lightest (Table 5).

3.2.4 Week 3 body weight

Bodyweight on day 21 was not significantly different between the five groups (mean values: 13866.11, 14056.9, 14000.5, 13480.73, 13221 for diet groups A, B, C, D and E, respectively. Notwithstanding this, (as in week 2) animals that received diets B and C continued to weighed more compared to those that fed the control diet while those that consumed diets D and E were still numerically lighter than the controls. The pattern of body weights was similar to week 1 and pigs that were offerd diet B regained the position of heaviest animals while those that consumed diet E maintain the position of lighest animals as in weeks one and two (Table 5).

In summary, animals that received diets B and C were heavier than the control while those that consumed diets D and E were lighter than the controls and remained light from week 2 to the end of the experiment. Diets B and C stimulated body weight growth better than the control diet while diets D and E depressed it.

3.2.5 Average daily gain (ADG)

Average daily gain is the total weight gained in a given period of time divided by the number of days (measured in g per day).

Significance of effects of wk0wt (body weight of pigs at the beginning of the study), diet, run, farm, sex, interaction between farm x diet ADG (average daily gain for the total period is shown in Annex 1).

Table 5 depicts least-square means of the effects of probiotic-supplemented diets on growth performance in weanling pigs for average pen live weight of piglets at the start of the experiment (Wk0wt), average pen live weight of piglets at the end of week 1 of the experiment (wk1wt), average pen live weight of piglets at the end of week 2 (wk2wt), average pen liv weight of piglets at the end of week 3 (wk3wt), average daily gain (ADG), average daily feed intake (ADFI) and average feed conversion rate (FCR) for the 21 d experimental period for each diet with standard error (SE).

Farm had an influence on ADG of pigs ($p = 0.0120$) (Annex 1). Bodyweight at weaning tendentiously affected ADG of pigs ($p=0.08$). However, average daily gain was not different ($p = 0.4093$) for piglets fed control diet (diet A) or probiotic-supplemented diets B, C, D and E (mean values: 305.41, 314.49, 311.80, 287.05 and 274.69 for diet A, B, C, D and E, respectively). Replica and sex had no effect on ADG of pigs and the interaction of farm x diet were not detected ($p>0.05$).

ADG follwed the pattern of feed intake and was numerically higher in pigs that consumed diets B and C compared to the controls and lower in pigs that recived diets D and E as will be observed below.

3.2.6 Average daily feed intake (ADFI)

The average daily feed intake is the total quantity of feed consumed in a given number of days divided by the number of days and number of animals consuming that feed (measured in g per animal per day).

The effect of diets on ADFI of pigs is depicted in Table 5.

The significance of effects of bodyweight at weaning, diet, replica, farm, sex, interaction of farm x diet on ADFI (average daily feed intake) are summarised in Annex 1.

There were significant ($p = 0.0169$) treatment differences for average daily feed intake in piglets fed control or probiotic B, C, D and E (mean values: 404.64, 426.77, 423.63, 378.48 and 341.48 for diet A, B, C, D and E, respectively). The effect of

bodyweight at weaning on ADFI of pigs was significant (p=0.0006) while replication had no effect on ADFI of pigs (p>0.05). Farm and sex tendentiously influenced ADFI for pigs (p=0.08 and p=0.06, respectively). An interaction of farm x diet was not detected (p>0.05) (Annex 1).

Feed intake in pigs that received diet B was significantly higher that in pigs that consumed the control diet (p=0.0169) while those in diet C ate more feed compared to the controls, but there was no signicant differences between the two groups (p>0.05). The pigs that received diets D and E consumed significantly low levels of feed compared to the controls (p<0.05).

To summarize, during the 21 day study period, animals that consumed diet B consumed significantly higher amounts of feed compared to the controls (p<0.05) while those in diet C group also ate more feed than those in the control group although the difference between the two groups was not significant (p>0.05). The pigs that were on diet D and E ate less feed than those given the control diet (p<0.05) [Table 5]. Diets B and C stimulated feed (energy) intake better than the control diet while diets D and E depressed it.

3.2.7 Average feed conversion ratio (AFCR)

Feed conversion ratio (FCR) is the ratio of feed eaten in a given period of time to the amount of weight gained over the same period of time. Since it is a ratio of parameters with same units (g) there are no units of measure.

The effect of diet on FCR of pigs is summarized in Table 5.

Significance of effects of bodyweight at weaning, diet, run, farm, sex, interaction between farm x diet on FCR (average feed conversion ratio) over the experimental period of 21 days are shown in Annex 1.

There were no treatment effects pigs (p=0.1843) on average feed conversion rate for piglets (p = 0.3776). Bodyweight at weaning had no effect on FCR of pigs (mean

values: 1.34299, 1.35963, 1.44081, 1.35278 and 1.2574 for diet A, B, C, D and E, respectively). Replica and sex did not affect FCR in pigs and the interaction of farm x diet were no significant ($p>0.05$) (Annex 1).

FCR was higher in pigs fed diets B, C and D compared to the controls while those in diet E tried to use the less quantity of feed ingested more efficiently compared to the controls (Table 5).

3.3 Effect of probiotic on Haematological (blood)parameters

3.3.1 Effect on whole blood parameters

Secondly, we investigated the effect of dietary treatment on haematological parameters. The blood samples collected on day 0, 14 and 21 of the experiment were used to analyse the effect on diet on the blood parameters and finally how performance and health are affected. To prevent double handling and to reduce stress, the pigs were weighed just before sampling.

The following is a list of parameters that were analysed: White blood cells (WBC), neutrophil cells (NEUT), lymphocyte cells (LYMPH), monocyte cells (MONO), eosinophil cells (EOS), basophil cells (BASO), red blood cells (RBC), haemoglobin concentration (HGB), haematocrit level (HCT), mean cell volume (or mean erythrocyte volume) (MCV), mean cell haemoglobin (or mean erythrocyte haemoglobin content)(MCH), mean corpuscular haemoglobin concentration (or mean erythrocyte haemoglobin concentration) (MCHC), corpuscular haemoglobin constant (CH), Corpuscular haemoglobin concentration mean (CHCM), corpuscular haemoglobin (CH), red blood cell distribution width (or erythrocyte distribution width)(RDW), haemoglobin distribution width (HDW), platelet (PLT), mean packed volume (MPV), neutrophil (NEUT), lymphocyte (LYMPH), monocyte (MONO), eosinophil (EOSI), and basophil (BASO).

Some whole blood parameters varied during the study. Most white cell population and differential counts (NEUT etc) in blood of pigs from different groups were within

normal range for pigs. Neither the control diet nor probiotic- nor lactoferrin-supplemeted diets altererd blood parameters ($p>0.05$) for the 21 day experimental, although some numerical differences were observed. WBC parameters showed showed little difference in levels and cellular responses to that of the animals fed the control diet, although they are reported and might be of some use as an indicator of disease or potential benefits (See Table 6 and Annex 1).

On the other hand, a few Red cell population and differential counts (RBC, HGB, HCT, MCH, etc) in blood of pigs from different groups were noticeably below normal range for pigs of that age group and blood parameters of pigs fed control or probiotic- or lactoferrin supplemeted diets did alter ($p>0.05$) for the 21 day experimental and some numerical differences were observed. Although most are reported, it is only clear that RBC, HCT, HGB and MCH were numerically lower in pigs fed diets D and E compared to the controls. It can also be seen that, the animals that consumed diets B and C had numerically higher levels of HCT and RBC than the controls (Table 6 and Annex 1). These body iron measures might be of some use as they can affect performance and health in pigs and will be discussed in detail.

3.3.1.1 Effect on white blood cell population parameters

For white blood cells we analysed: White blood cells (WBC), neutrophil cells (NEUT), lymphocyte cells (LYMPH), monocyte cells (MONO), eosinophil cells (EOSI) and basophil cells (BASO).

3.3.1.1.1 Absolute Leukocyte cells or white blood cells

Statistical significance of effects of farm, diet, day and their interactions on white blood cell parameters are presented in Annex 1.

Table 6 depicts the effects of probiotic-supplemented diets on leukocyte or WBC (white blood cell) components in weanling pigs.

The effect of farm on WBC counts in pigs was significant ($p=0.0104$). Repeated measures of analysis revealed that WBC counts in pigs were unaffected by feeding control or probiotic-supplemented diets ($p=0.2609$) as there were no statistically significant differences between the five diet groups (means values: 15.97, 17.81, 16.46, 17.35 and 16.31 for diet A, B, C, D and E, repectively), although WBC varied

($p<0.0001$) over time in all diet groups. WBC increased significantly ($p<0.05$) from day 0 to peak on day 14 and declined (slightly) from day 14 to 21. The decrease was significant only in pigs fed diet A and E. Interactions of farm x diet, farm x day, farm x diet x day and diet x day ($p>0.05$) were not found.

Table 6: Effects of probiotic- and lactoferrin-supplementation on whole bood parameters and blood cell parameter changes in weaned piglets

Parameter		A (control)	B (probiotic B)	C (probiotic B)	D (probiotic B)	E (lactoferrin)	SE	pvalue
Effect on whole blood parameters								
WBC	day							
0		11.88[a]	12.1[a]	12.14a	12.24[a]	12.02[a]	0.72	0.4289
(x 10^9	day 14	18.16[b]	20.75[b]	18.92[b]	20.23[b]	19.5[b]	0.72	
Cells/L)	day 21	17.32[c]	20.57[b]	18.33[b]	19.58[b]	17.42[c]	0.72	
	mean value	15.97	17.81	16.46	17.35	16.31	0.42	
Neutrophil (x 10^9 cells/L)	Day 0	4.56[a]	5.75[a]	5.13[a]	5.07[a]	5.51[a]	0.5	0.2708
	day 14	8.31[c]	9.49[c]	8.69[b]	9.67[c]	9.06[c]	0.5	
	day 21	7.14[b]	8.68[b]	5.13[a]	8.92[b]	7.52[b]	0.5	
	mean value	6.67	7.97	7.04	7.89	7.36	0.29	
Lymphocytes	Day 0	6.42[a]	5.45[a]	6.1[a]	6.17[a]	5.65[a]	0.4	0.6342
(x 10^9	day 14	8.48[b]	9.58[b]	8.84[b]	9.06[b]	8.9[c]	0.4	
cells/L)	day 21	9.03[c]	10.19[c]	9.2[b]	9.21[b]	8.4[b]	0.4	
	mean value	7.97	8.41	8.07	8.15	7.68	0.23	
monocyte (x 10^9 cells/L)	Day 0	0.62[a]	0.52[a]	0.48[a]	0.05[a]	0.49[a]	0.11	0.3711
	day 14	0.81[b]	0.99[b]	0.75[b]	0.8[b]	1.28[b]	0.11	
	day 21	0.73[ab]	1.05[b]	0.88[c]	0.82[b]	0.74[c]	0.11	
	mean value	0.72	0.85	0.7	0.71	0.84	0.06	
Eosinophil	Day 0	0.19[a]	0.22[a]	0.19[a]	0.24a	0.18[a]	0.03	0.6002
(x 10^9	day 14	0.29[b]	0.32[b]	0.34[b]	0.36[b]	0.33[b]	0.03	
cells/L)	day 21	0.3[c]	0.33[b]	0.4[c]	0.33[b]	0.38[c]	0.03	

	mean value	0.26	0.29	0.31	0.31	0.3	0.02	
Mean Corpuscular Volume (fL)	Day 0	54.13[a]	54.99[a]	54.87[a]	53.81[a]	55.26[a]	0.76	0.9307
	day14	52.24[b]	50.98[b]	51.69[b]	50.5[b]	51.15[b]	0.76	
	day21	52.83[b]	51.8[c]	52.48[c]	50.96[b]	51.9[c]	0.76	
	mean value	52.59	52.59	53.01	51.76	52.79	0.44	
Mean corpuscular haemoglobin (pg)	Day 0	18.03[a]	17.79[a]	17.91[a]	17.5[a]	17.8[a]	0.2	0.939
	day 14	16.97[b]	16.42[b]	16.71[b]	16.48[b]	16.5[b]	0.2	
	day 21	16.78[b]	16.44[b]	16.68[b]	16.33[b]	16.6[b]	0.2	
	mean value	17.3	17	17	16.8	17	0	
MCHC (g/L)	day 0	321.58	322.6	321.5	323.2	320	1.2	
	day 14	324.21	321.5	323	325.2	321.3	1.2	
	day 21	317.44	313.7	317.7	319.3	318.1	1.2	
	mean value	321.08	319.3	320.7	322.6	319.8	2.1	
CHCM (g/L)	day 0	311.65[a]	312[a]	309.75[a]	311.09[a]	309.88[a]	1.47	0.9625
	day 14	316.5[b]	315.59[b]	313.91[b]	318.13[b]	315.13[b]	1.47	
	day 21	307.78[c]	307.05[c]	306.06[c]	308.5[c]	307.75[c]	1.47	
	mean value	311.98	311.6	309.9	312.6	310.9	0.85	
RDW (%)	day 0	22.27	23.42	23.5	24.02	23.75	0.35	
	day 14	22.35	24.13	23.15	23.2	23.5	0.35	
	day 21	21.54	23.12	22	22.3	22.73	0.35	
	mean value	22.06	23.56	22.88	23.18	23.33	0.2	
Effect on blood cell parameters changes								
WBC (x 10^9 cells/L)	d14-d0	6.41	8.52	6.78	7.99	7.5	0.71	0.2676
	d21-d0	5.31	8.56	6.19	7.34	5.39	0.71	
	Trial mean	5.86	8.56	6.49	7.66	6.44	0.5	
NEUT, x 10^9 cells/L	d14-d0	3.8	3.66	3.56	4.56	3.61	0.46	
	d21-d0	2.53	3.01	2.17	3.88	2.01	0.46	
	Trial mean	3.16	3.34	2.87	4.22	2.81	0.33	
LYMPH, x 10^9 cells/L	d14-d0	2.12	4.1	2.69	2.92	3.22	0.38	
	d21-d0	2.54	4.77	2.08	3.02	2.85	0.38	

	Trial mean	3.16	3.34	2.87	4.22	2.81	0.33
MONO, x 10^9 cells/L	d14-d0	0.18	0.46	0.27	0.29	0.79	0.13
	d21-d0	0.11	0.55	0.41	0.31	0.25	0.13
	Trial mean	0.15	0.51	0.34	0.3	0.52	0.09
EOSI, x 10^9 cells/L	d14-d0	0.1	0.1	0.15	0.12	0.16	0.03
	d21-d0	0.11	0.1	0.21	0.09	0.2	0.03
	Trial mean	0.1	0.1	0.18	0.11	0.18	0.02
BASO, x 10^9 cells/L	d14-d0	0.01	0.07	0.01	0.06	0.04	0.01
	d21-d0	0.01	0.07	0.04	0.04	0.03	0.01
	Trial mean	0.01	0.07	0.02	0.05	0.04	0.01
RBC, x 10^6 cells/L	d14-d0	0.33	0.43	0.36	0.44	0.54	0.08
	d21-d0	0.08	0.29	0.37	0.2	0.24	0.08
	Trial mean	0.21	0.35	0.37	0.32	0.39	0.05
HGB (g/L)	d14-d0	-0.41	-1.82	-1.66	3.12	-0.1	1.79
	d21-d0	-6.43	-7.51	-1.75	-1.89	-5.3	1.79
	Trial mean	-3.42	-4.67	-1.7	0.62	-2.7	1.26
HCT	d14-d0	-0.002	0.002	-0.004	0.002	0.001	0
	d21-d0	-0.015	-0.002	-3E-04	-0.008	-0.009	0
	Trial mean	-0 0086	0.0004	-0.002	-0.003	-0.004	0
MCV (fL)	d14-d0	-1.83	-4.03	-3.18	-3.3	-4.1	0.38
	d21-d0	-1.35	-3.16	-2.39	-2.87	-3.3	0.38
	Trial mean	-0 009	0.0004	0	0	0	0.003
MCH (pg)	d14-d0	-1.05	-1.35	-1.2	-1	-1.3	0.15
	d21-d0	-1.25	-1.36	-1.23	-1.2	-1.2	0.15
	Trial mean	-1.15	-1.36	-1.21	-1.1	-1.3	0.11
MCHC (g/L)	d14-d0	2.54	-0.62	1.5	2.3	1.48	2.19
	d21-d0	-4.05	-9.36	-3.78	-4.27	-2	2.19
	Trial mean	-0.75	-4.99	-1.14	-0.99	-0.3	1.55
CHCM (g/L)	d14-d0	4.73	3.81	4.16	7.01	5.63	1.33
	d21-d0	-3.74	-5.16	-3.69	-2.48	-2.1	1.33
	Trial mean	0.49	-0.67	0.23	2.27	1.75	0.94
RDW (%)	d14-d0	-0.1	0.7	-0.35	-0.82	-0.2	0.3
	d21-d0	-0.55	-0.29	-1.5	-1.71	-1	0.3
	Trial mean	-0.32	0.2	-0.93	-1.27	-0.1	0.22

[a,b c] values in the same column with different letters implies that from this day to the next the MCV levels are significantly different ($p<0.05$).

SE standard error

3.3.1.1.2 Neutrophils

Statistical significance of effects of farm, diet, day and their interactions on neutrophils cells are presented in Annex 1.

The least square means of values for neutrophils ($x10^9$ cells/L) obtained from each diet treatment are shown in Table 6.

NEUT counts in pigs were affected ($p<0.0001$) by time and were lowest on day 0, peaked on day 14 and reduced thereafter from day 14 to 21. Effect of farm on neutrophil counts in pigs was significant ($p=0.0014$). However, neutrophil cell counts were not different for piglets fed the control or the probiotic supplemented diets ($p = 0.2708$). Interactions of farm x diet, farm x diet x day and diet x farm were not detected ($p>0.05$).

As can be observed from in Table 6, the neutrophil cells increased in all diet groups from day 0 to 21 with the highest being in pigs fed diet D (from 5.07 to 8.92). All diets showed an increase from week 0 to week 2 with diet D showing the highest increase (5.07 to 9.67). From day 14 to 21, the neutrophil counts in pigs in all diets groups declined with pigs fed diet E declining at a faster rate (from 9.06 to 7.42). The NEUT cell counts of the pigs on day 14 and day 21 were numerically lower (8.31 and 7.14, respectively) for the control (diet A) and continued to be low throughout the trial period. When the four treatments under investigation (diet B, C, D and E) were compared, feeding probiotic in diet B resulted in pigs achieving higher neutrophil cell count (9.49 and 8.68 in day 14 and day 21, respectively) than the probiotic in diet E.

Probiotic supplementation stimulated NEUT in pigs more compared to the control diet. Stimulation was highest in pigs that consumed diet B and least in those given diet C.

NEUT varied with time ($p<0.0001$). In pigs fed the control diet, NEUT increased from 4.56 x 10^9 cells/L at day 0 (28 days) to 8.31 x 10^9 cells/L at day 14 (42 days). From day 14 (42 day), the levels decreased to 7.14 8.31 x 10^9 cells/L at day 21 (49 days old) (Table 6).

3.3.1.1.3 Lymphocytes

Annex 1 shows the statistical significance of effects of farm, diet, day and their interactions on lymphocytes of pigs.

Effects of probiotic-supplemented diets on lymphocytes in weanling pigs are presented in Table 6.

Lymphocyte counts were affected ($p<0.0001$) by time and were lowest on weaning day, increased and peaked on day 14 and reduced thereafter from day 14 to day 21. Farm had an influence on lymph counts ($p<0.0001$). An interaction of farm x day ($p=0.0038$) was evident. Lymphocyte counts were not different for pigs fed diet A (control) or the probiotic-supplemented diets (B, C, D and E) ($p = 0.6342$) although the changes in LYMPH during the period from day 0 to 14 and period from day 0 to 21 in pigs fed the control and probiotic-supplemeted diets tended to be different ($p=0.08$) (Table 6). The interactions of farm x diet, farm x diet x day and diet x day were not detected ($p>0.05$).

3.3.1.1.4 Monocytes

Annex 1 depicts the statistical significance of effects of farm, diet, day and their interactions on monocyte cells of pigs.

The least square means of values for monocyte cells ($x10^9$ cells/L) obtained from each diet treatment are presented Table 6.

Monocyte cell numbers in pigs were affected ($p<0.0001$) by time and were lowest on day 0. Farm had an influence on monocyte cell couts ($p=0.0159$) (Annex 1). Nevertheless, least-square mean of monocyte counts were not different for piglets fed the control or probiotic-supplemeted diets (B, C, D and E) ($p = 0.3711$). There was a trend towards an interactions of diet x day ($p=0.10$). However, interactions of farm x diet, farm x day and farm x diet x day were not detected ($p>0.05$).

3.3.1.1.5 Eosinophils

Annex 1 gives the statistical significance of effects of farm, diet, day and their interactions on eosinophil cells blood parameters of pigs.

The least square means of values for eosinophil cells ($x10^9$ cells/L) obtained from each diet treatment are presented in Table 6.

Eosinophil cell counts in pigs were affected ($p<0.0001$) by time and were lowest on weaning day. Farm had an influence on EOSI counts in pigs ($p=0.0007$). However, dietary supplementation with probiotic B, C, D and E had no effect on eosinophils in pigs ($p = 0.6002$). There was a trend towards an interaction of farm x day ($p=0.06$) although there were no interactions of farm x diet, farm x diet x day and diet x day ($p>0.05$).

Overall, trial LSMeans eosinophils counts for piglets fed the control and probiotic-supplemented diets for the duration of the study were 0.26, 0.29, 0.31, 0.31 and 0.3 for diet A, B, C, D and E, respectively (Table 6). Overall, EOSI increased in pigs in all diet groups from day 0 to 21. Trial means or the least-square means for the increase in EOSI were 0.1, 0.1, 0.18, 0.11 and 0.18 for diet A, B, C, D and E, respectively (Table 6). In our study the eosinophil counts increased. The increase was higher in animals in the diet C group than in control group and lowest in those in diet B group (0.18, 0.1 and 0.1, respectively).

As can be observed from Table 18, the eosinophil cell counts in pigs increased for all diet groups from day 0 to 21. The eosinophil cells of pigs that consumed diets A, B, C and E continued to increase throughout the experimental period apart from the eosinophil cells of pigs fed diet D that increased from day 0 and reached the peak (0.33) on day 14 and on day 21 it was lower (0.13×10^9 cells/L). When the four treatments under investigation (diet B, C, D and E) were compared, feeding probiotic in diet C resulted in pigs achieving higher eosinophil cell count (0.34 and 0.4) on day 14 and 21, respectively) than the probiotic in other diets over the 3 week study. This

was evidenced by a relatively high increase in number of eosinophil cells in pigs that consumed diet B for the duration of the study.

Probiotic treated diets stimulated eosinophil cellsin pigs more than in the control diet throughout the study period. Stimulation was higher in pigs fed diet C compared to B among the treatment groups.

3.3.1.1.6 Basophils

Annex 1 depicts the statistical significance of effects of farm, diet, day and their interactions on basophil cells of pigs.

The least-square means of values for basophil cells ($x10^9$ cells/L) obtained from each diet treatment are summarized in Table 6.

Basophil counts in pigs were affected (p=0.0012) by time and were lowest on weaning day and an interaction of farm x day (p=0.0315) was detected (Table 6). Treatment means for basophil cell counts did not differ among treatment (diet) groups (p = 0.4762). The effect of farm on basophil counts of pigs was not significant (p=0.5122) and interactions of farm and diet, farm x diet x day and diet x day were not found (p>0.05).

Overall, Diet B and C had higher basophils than control while diet D and E group had lower basophils than control.

As can be observed from in Table 20, from weaning day (day 0) to day 14, the basophil cell counts increased for all diet groups apart from diet C that remained unchanged from weaning day to day 14. (0.1 and 0.1, respectively). When we compare cell counts from weaning day to day 14 and from weaning day to day 21, the cell counts of all diets are lower from weaning day to day 21 except for diet C that had a higher cell count from weaning day to day 21 compared from weaning day to day 14 (0.1 and 0.14, respectively). Cell counts control group were similar in periods between day 14 and weaning day and day 21 and weaning day (0.11 and 0.11, respectively).

Suplementation with probiotic B and C stimulated basophil cells more compared to control while stimulation by diet D and E was lower than in the control.

As can be observed from in Table 6, the basophil cell counts increased for all diet groups from weaning day to 21. The basophil cells of pigs fed diet E remained numerically lower throughout the 21 d period. Pigs that consumed diet C remained unchanged from day 0 to 14 but showed a rapid increase from day 14 onwards. Pigs fed the control diet remained unchanged from weaning day to day 14 but showed a very small increase from day 14 onwards. Pigs that consumed diets D and E showed rapid rise in this cell from weaning day to day 14 but showed a decline in BASO from day 14 onwards with pigs offered diet D showing a steeper slope of decline compared to those that consumed diets B and E. When the four probiotics under investigation (diet B, C, D and E) are compared, feeding probiotic in diet C resulted in pigs achieving highest basophil cell count (0.1 and 0.14 on weaning day and day 21, respectively) than the pigs supplemented with probiotic in diet E. This is evidenced by the highest rise in number of basophil from day 14 onwards.

3.3.1.2 Erythrocytes or red blood cell populations and red cell indices parameters

Red blood cells types analysed included red blood cells (RBC), haemoglobin concentration (HGB), haematocrit level (HCT), mean cell volume (or mean erythrocyte volume) (MCV), mean cell haemoglobin (or mean erythrocyte haemoglobin content)(MCH), mean corpuscular haemoglobin concentration (or mean erythrocyte haemoglobin concentration) (MCHC), corpuscular haemoglobin constant (CH), Corpuscular haemoglobin concentration mean (CHCM), corpuscular haemoglobin (CH), red blood cell distribution width (or erythrocyte distribution width)(RDW), haemoglobin distribution width (HDW), platelet (PLT), mean packed volume (MPV), neutrophil (NEUT), lymphocyte (LYMPH), monocyte (MONO), eosinophil (EOSI), and basophil (BASO).

3.3.1.2.1 Erythrocytes or red blood cells

Statistical significance of effects of farm, diet, day and their interactions on red blood cell parameters are presented in Annex 1.

Table 6 depicts the effects of probiotic-supplemented diets on blood components in weanling pigs.

RBC counts of pigs were affected ($p=0.0008$) by time. RBC increased in all groups from day 0 to day 14 ($p<0.05$) and decreased in all diet groups from day 14 to 21. The decrease was significant ($p<0.05$) in all diet groups except in pigs that consumed diet C. They were highest on day 14 and reduced from day 14 to day 21 except in diet C were they increased slightly (by 0.1 x 10^9cells/L). The effect of farm on RBC counts of pigs was significant ($p=0.0004$). The interactions of farm x diet and farm x day were significant ($p=0.0103$, and $p=0.0032$, respectively) [data not shown] while, interactions of farm x diet x day and diet x day ($p>0.05$) were not detected (Table 12). Dietary supplementation with control (diet A) and probiotic-supplemented diets had no significant effect ($p=0.4289$) on RBC counts of pigs (means values: 5.93, 5.99, 5.96, 5.87 and 5.75 for diet A, B, C, D and E, respectively).

RBCs carry oxygen from lungs to cells and CO_2 from cells back to the lungs. High levels of RBCs is an indicator of good health. Pigs were randomly allocated to the five diet groups but by chance those that were in groups D and E had lower RBC levels compared to other groups. This was not expected. As the study progressed, animals that consumed these two diets (D and E) still remained with the lowest RBC levels compared to pigs in other diet groups. At the end of the study, pigs fed diets D and E had lower RBC levels compared to the controls while those that consumed diets B and C had higher RBC than the controls.

3.3.1.2.2 Haemoglobin concentration

Annex 1 shows the statistical significance of effects of farm, diet, day and their interactions on haemoglobin concentration in pigs.

Effects of probiotic-supplemented diets on haemoglobin concentration in weanling pigs are tabled in Table 6.

Farm had a significant effect on HGB of pigs ($p<0.0001$). An interaction of farm x day ($p =0.0397$) was observed for RBC count in pigs. As shown in Table 6, haemoglobin levels were not different ($p=0.6021$) for piglets fed the control or the probiotic-supplemented diets (mean values: 101.89, 100.14, 101.14, 101.37, 96.85 and 98.16 for diet A, B, C, D and E, respectively). HGB levels did not vary over time ($p=0.2129$). Interactions of farm x diet, farm x diet x day and diet x day were not detected ($p>0.05$.

3.3.1.2.3 Haematocrit or packed cell volume or volume of packed red cells

Haematocrit (HCT) or packed cell volume (PVC) is the percentage of erythrocytes or RBC in whole blood. Low HCT is an indicator of late stage anaemia (Thrall *et al.*, 2004; Thompson & Forsyth, 2006; Tvedten, 1993 and Voigt, 2000).

Annex 1 depicts the statistical significance of effects of farm, diet, day and their interactions on haematocrit measures of pigs.

Table 6 shows the effects of probiotic-supplemented diets on haematocrit measures in weanling pigs.

Farm had a significant influence on haematocrit measures in pigs ($p=0.0002$). An interation of farm x day ($p=0.0088$) was noted for HCT. Nevertheless, no statistical differences ($p = 0.3324$) were observed on HCT of pigs by addition of probiotics or lactoferrin on haematocrit between the diet groups (mean values: 0.317, 0.313, 0.315, 0.302 and 0.303 for diet A, B, C, D and E, respectively). Interactions of farm x diet,

farm x diet x day were not detected (p>0.05). HCT measures were not affected by time (p=0.6181) (Annex 1).

HCT or PCV can be used as an indicator of anaemia in animals. High levels indicate high iron levels whereas low is an indicator of ill health. As reported for RBCs and HGB above, piglets were randomly allotted into the five diet groups but by chance those that were in groups D and E had lower HCT levels compared to other groups. This was, again, not expected. Overall, between weaning day and day 21, pigs that consumed diet D and E had lower levels of HCT compared to the controls. On the other hand, pigs that consumed diets B and C had higher levels of HCT compared to the controls.

3.3.1.2.4 Mean corpuscular volume

Erythrocyte volume is the MCV. In other words, MCV is an average volume of a single cell of the red blood cells measured in femtolitres, fL [1 fL = 10^{-15} L or 1μL = 10^{9} fL] (Thrall et al., 2004; Thompson & Forsyth, x; Tvedten, 1993; Weiser, x; Bush, x). MCV may be used as a morphological indicator of iron deficiency since this value gives a description of the normality of red blood cell size.

Annex 1 shows the statistical significance of effects of farm, diet, day and their interactions on blood parameters of pigs.

The effects of probiotic-supplemented diets on blood components in weanling pigs are summarized in Table 6.

MCV of pigs were affected (p=0.0195) by time. MCV decreased (p<0.05) in pigs in all diet groups from day 0 to 14. From day 14 to 21, MCV increased in pigs in all diet groups although the increase was significant (p<0.05) only in pigs that were offered diets B, C and E. Farm had an influence on MCV counts of pigs (p=0.0013). Trends (p = 0.9307) were observed on MCV of pigs between the diet groups (mean values: 53.06, 52.59, 53.01, 51.76 and 52.79 for diet A, B, C, D and E, respectively). Interactions of farm x diet and farm x diet x day, farm x diet x day and diet x farm were not evident (p>0.05).

3.3.1.2.5 Mean corpuscular haemoglobin

MCH is calculated by dividing the amount of HGB (g/L) by the RBC count [$x10^{12}$ cells/L] (Thrall *et al.* 2004; Thompson & Forsyth, 2006; Tvedten, 1993).

Annex 1 shows the statistical significance of effects of farm, diet, day and their interactions on blood parameters of pigs.

The effects of probiotic-supplemented diets on MCH in weanling pigs are presented in Table 6.

Farm had an influence on MCH of pigs ($p<0.0001$). MCH varied over time ($p=0.0123$). From day 0 to 14, MCH decreased significantly ($p<0.05$) in pigs in all diet groups. From day 14 to 21, MCH further declined ($p>0.05$) in pigs that consumed diets A, C and D and increased ($p>0.05$) in those that received diet B and E.
Statistically no signicant differences ($p = 0.9390$) in MCH measures were observed in pigs fed control and probiotic-supplemented diets (mean values: 17.26, 16.88, 17.1, 16.77 and 16.97 for diet A, B, C, D and E, respectively).
There were no significant interactions of farm x diet, farm x day; farm x diet x day and diet x day ($p>0.05$).

3.3.1.2.6 Mean corpuscular haemoglobin concentration

The amount of haemoglobin within erythrocyte (MCHC) is calculated as HGB (g/dL) divided by PCV (ml/100ml) x 100 = MCHC.
Statistical significance of effects of farm, diet, day and their interactions on MCHC of pigs are summarized in Annex 1.

Table 6 depicts the statistical significance of effects of farm, diet, day and their interactions on MCHC of pigs.

There was an influence of farm on MCHC of pigs (p=0004). MCHC were not affected by time (p=0.2218) and no significant differences (p = 0.9629) were observed in MCHC between the diet groups. Interactions of farm x diet, farm x day, farm x diet x day and diet x day were not detected (p>0.05) (Table 6).

3.3.1.2.7 Corpuscular haemoglobin concentration mean

Annex 1 depicts the statistical significance of effects of farm, diet, day and their interactions on corpuscular haemoglobin concentration mean (CHCM) of pigs.

Effects of probiotic-supplemented diets on corpuscular haemoglobin concentration mean in weanling pigs are shown in Table 6.

CHCM of pigs was affected (p<0.0149) by time. From day 0 to 14, CHCM increased (p<0.05) in pigs in all diet groups. From day 14 to 21, CHCM in all the pigs declined (p<0.05). Farm had an influence on CHCM counts in pigs (p<0.0001) (Annex 1). However, no significant differences (p = 0.9625) were observed between diet groups in CHCM in pigs by addition of probiotics (B, C, D and E) and interactions of farm x diet, farm x diet x day, farm x day, diet x day were not evident (p>0.05).

3.3.1.2.8 Red cell distribution width

Annex 1 depicts the statistical significance of effects of farm, diet, day and their interactions on red cell distribution width of pigs.

The least square means values for red cell distribution width (%) obtained from each diet treatment are presented in Table 6.

Farm had an influence on RDW of pigs (p < 0.0001) (Annex 1). However, no significant differences (p = 0.7627) were observed by addition of probiotics (B, C, D and E) on RDW of pigs. No interactions of farm x diet and farm x diet x day, diet x

day and farm x day were detected (p>0.05). RDW in pigs did not vary with time (p>0.05).

3.3.2 Blood cell parameter changes

Thirdly, we investigated the effect of dietary treatment on blood changes. Blood samples were taken on day weaning day, day 14 and 21 of the study period. Changes were obtained by subtracting blood counts on weaning day from that of day 14 (d14 – d0) and that of day 21 (d21-d0).

3.3.2.1 Absolute white blood cell changes

Under white blood cell changes, we analysed WBC, NEUT, LYMP, MONO, EOSI, BASO between day 14 and 0 (d14-0) and day 21 and 0 (d21-0).

3.3.2.1.1 Leukocyte or white blood cell changes

Statistical significance of effects of farm, diet, day and their interactions on white blood cell changes are presented in Annex 1. There was an influence of farm on WBC changes of pigs (p=0.0056), while time had no significant effect on WBC (p=0.2986). LSMeans for WBC changes were not different for piglets fed the control or the probiotic-supplemented diets (p = 0.2679). Interactions of farm x diet, farm x day, farm x diet x day and diet x day were not observed (p>0.05).

3.3.2.1.2 Neutrophil cell changes

The statistical significance of effects of farm, diet, day and their interactions on neutrophil cells are presented in Annex 1.
Least-square means of neutrophil cells of each treatement is shown in Table 6.
Farm had an effect on neutrophil cell changes of pigs (p=0.0381). Neutrophil changes tendentiously varied over time (p=0.06) and were highest on day 14 post weaning. However, no significant differences (p=0.5769) were observed in pigs fed control and probiotic-supplemented diets on neutrophil cell changes. Interactions of farm x diet, farm x day, diet x day, farm x diet x day were not evident (p>0.05).

From the start of the experiment to the end of week 2 (d0 to d14) least-square means of neutrophil cell increase were 3.8, 3.66, 3.56, 4.56 and 3.61 for diet A, B, C, D and E, respectively.

From the start of the experiment to the end of week 3 (d0 to d21) least-square means of neutrophil cell increase were 2.53, 3.01, 2.17, 3.88 and 2.01 for diet A, B, C, D and E, respectively.

Overall, least-square means of neutrophil cell changes increased by 3.16, 3.34, 2.87, 422 and 2.81 for diet A, B, C, D and E, respectively.

From day 0 to 14, NEUT cells increased in pigs fed control and probiotic-supplemented diets. However, from day 14 to 21, pigs in all diet groups showed areduction in NEUT cells.

As can be observed from Table 6 and Annex 1 changes tended to vary with time ($p=0.06$). Least-significant means for NEUT change during the period from day 0 to 14 was 3.8×10^9 cells/L for the control animals. For the period from 0 to 21 these NEUT change was 2.53×10^9 cells/L.

3.3.2.1.2 Lymphocyte cell changes

The statistical significance of effects of farm, diet, day and their interactions on lymphocyte cell changes are presented in Annex 1.
Least-square means of lymphocyte cells of each treatement is shown in Table 6.

Farm effect had an influence on lymphocyte cells changes of pigs ($p<0.0001$). Lymphocyte cell changes tended to be different in pigs fed control and probiotic-supplemented diets ($p=0.08$). Lymphocyte counts in pigs fed control and treatment diet were not affected by time ($p>0.05$) and the interactions of farm x diet, farm x day, diet x day, farm x diet x day were not observed ($p>0.05$).

From the start of the experiment to the end of week 2 (d0 to d14) least-square means of lymphocyte cells increase were 2.12, 4.1, 2.69, 2.92 and 3.22 for diet A, B, C, D and E, respectively

The increase in lymphocytes was higher in pigs in the treated groups compared to the controls. Among the treated groups pigs that were fed diet B had the highest increase while those that received diet C had the lowest increase.

From the start of the experiment to the end of week 3 (d0 to d21) least-square means of lymphocyte cells increase were 2.54, 4.77, 2.08, 3.02 and 2.85 for diet A, B, C, D and E, respectively. The increase in lymphocytes was higher diet B, D and E groups compared to the control while diet C had a smaller increase compared to control. Among the treated groups diet B had the highest.

Overall, least-square means of lymphocyte cell changes increase were 3.16, 3.34, 2.87, 4.22 and 2.81 for diet A, B, C, D and E, respectively.

From week 0 to week 2 (d14 - d0), lymphocyte cells in pigs receiving diet A had numerically the smallest increase of 2.12×10^9 cells/L while diet B had the highest increase (4.1×10^9 cells/L).

As seen in Table 6, the LSMeans for the increase in lymphocyte cell during the period from 0 to 21d was highest in diet B(4.77×10^9 cells) and was lowest in diet C (2.08×10^9 cells).

Lymphocyte proliferation tended to be higher in pigs that consumed (probiotic- and lactoferrin-) supplemented diets than the control diet and tended to higher in diets B and lower in diets C, D and E ($p=0.08$). Changes in lymphocytes proliferation tended to be higher in pigs that received diet B.

As can be observed from Table 6 and Annex 1 LYMPH changes tended to vary with time ($p=0.06$). Least-significant means for LYMPH change during the period from

day 0 to 14 was 2.12 x 10^9 cells/L for the control animals. For the period from 0 to 21 these LYMPH change was 2.54 x 10^9 cells/L.

3.3.2.1.3 Monocyte cell changes

The statistical significance of effects of farm, diet, day and their interactions on monocyte cell changes are depicted in Annex 1.

Least-square means of monocyte cell changes of each treatement is shown in Table 6.

Farm tended to affected monocyte cell changes in pigs ($p=0.09$) although the monocyte changes were not affected by time ($p>0.05$).
No significant differences ($p=0.1870$) were observed in pigs fed control and probiotic-supplemented diets on monocyte cell changes. Interactions of farm x diet, farm x day, diet x day, farm x diet x day were not detected ($p>0.05$).

3.3.2.1.4 Eosinophil cell changes

The statistical significance of effects of farm, diet, day and their interactions on eosinophil cell changes are presented in Annex 1.

Least-square means for effects of probiotic-supplemented diets on eosinophil changes in weanling pigs are shown in Table 6.

There was an effect of farm on eosinophil cells changes of pigs ($p=0.0008$). No significant differences ($p=0.3790$) were observed in pigs fed control and probiotic-supplemented diets on eosinophil cell changes. EOSI changes were not affected by time and interactions of farm x diet, farm x day, diet x day, farm x diet x day were not found ($p>0.05$).

3.3.2.1.5 Basophil cell changes

The statistical significance of effects of farm, diet, day and their interactions on basophil cell changes are presented in Annex 1.

Least-square means for effects of probiotic-supplemented diets on basophil changes in weanling pigs are shown in Table 6.

Farm had an effect on basophil cell changes in pigs ($p=0.0005$). However, no significant differences ($p=0.1974$) were observed in pigs fed control and probiotic-supplemented diets on basophil cell changes. Eosinophil cell changes were not affected by time ($p>0.05$). Interactions of farm x diet, farm x day, diet x day, farm x diet x day were not noted ($p>0.05$).

3.3.2.2 Erythrocyte or red blood cell changes

Under red red blood cell changes, we analysed: red blood cells (RBC), haemoglobin concentration (HGB), haematocrit level (HCT), mean cell volume (or mean erythrocyte volume) (MCV), mean cell haemoglobin (or mean erythrocyte haemoglobin content)(MCH), mean corpuscular haemoglobin concentration (or mean erythrocyte haemoglobin concentration) (MCHC), corpuscular haemoglobin constant (CH), Corpuscular haemoglobin concentration mean (CHCM), corpuscular haemoglobin (CH), red blood cell distribution width (or erythrocyte distribution width)(RDW), haemoglobin distribution width (HDW), platelet (PLT), mean packed volume (MPV) between day 14 and 0 (d14-0) and day 21 and 0 (d21-0).

3.3.2.2.1 Erythrocyte or red blood cell changes

Statistical significance of effects of farm, diet, day and their interactions on red blood cell changes are presented in Annex 1.

Effect of diet on RBC is depicted in Table 6.

Farm had a significant influence on red blood cells of pigs ($p < 0.001$). LSMeans for red blood cell changes was not different for piglets fed the control or the probiotic-supplemented diets ($p = 0.8791$) and there were no interactions observe ($p>0.05$).

3.3.2.2.2 Haemoglobin changes

Annex 1 depicts the statistical significance of effects of farm, diet, day and their interactions on blood HGB of pigs.

Effect of diet on HGB is shown in Table 6.

Farm had an influence on haemoglobin of pigs ($p < 0.0001$) while HGB in pigs did not vary with time ($p=0.1082$). LSMeans for haemoglobin changes were not different for piglets fed the control or the probiotic-supplemented diets ($p = 0.7947$) and interactions of farm x diet, farm x day, diet x day and farm x diet x day were not evident ($p>0.05$).

Overall, least-square means of HGB decrease/increase were -3.42, -4.67, -1.7, 0.62 and -2.69 g/L for for diet A, B, C, D and E respectively. Pigs in diet D group, however showed an increase of HGB by 0.62 g.

The decrease in HGB was lower in pigs fed diet C, D (increase) and E probiotic-supplemented groups compared to the controls while pigs fed diet B had a higher decrease than the controls. Among treatments, diet D pigs had an increase in HGB while those fed diet E had a higher decrease.

The decrease in pigs that received diet D was always numerically lower than controls while the decrease in pigs fed diet B was always numenrically higher than control animals.

3.3.2.2.3 Haematocrit changes

Annex 1 depicts the statistical significance of effects of farm, diet, day and their interactions on blood changes parameters of pigs.

Least square means for effects of probiotic-supplemented diets on haematocrit changes in weanling pigs are summarized in Table 6.

Farm had an effect on HCT of pigs ($p = 0.0001$) while HCT did not vary with time ($p=0.4088$). LSMeans for HCT was not different for piglets fed the control or the probiotic-supplemented diets ($p = 0.9694$) and interactions of farm x diet, farm x day, diet x day and farm x diet x day were not noted ($p>0.05$).

3.3.2.2.4 Mean corpuscular volume changes

Statistical significance of effects of farm, diet, day and their interactions on mean corpuscular volume changes parameters of pigs are presented in Annex 1.

LSMeans for mean corpuscular volume levels was not different for piglets fed the control or the probiotic-supplemented diets ($p = 0.7579$) and interactions of farm x diet, farm x day, diet x day and farm x diet x day were not observed ($p>0.05$). Farm had no effect on MCV levels of pigs and MCV of pigs did not vary with time ($p>0.05$).

3.3.2.2.5 Mean corpuscular haemoglobin changes

Annex 1 gives the statistical significance of effects of farm, diet, day and their interactions on mean corpuscular haemoglobin of pigs.

Farm had a significant effect on MCH of pigs (p=0.0030) while time tended to have an effect on MCH of pigs (p=0.7722) and LS Means for MCH levels was not different for piglets fed the control or the probiotic-supplemented diets (p = 0.9735). Interactions of farm x diet, farm x day, diet x day and farm x diet x day were not observed (p>0.05).

Least square means for mean corpuscular haemoglobin changes are presented in Table 6.

From day 0 to 14 (d14 - d0), MCH decreased in all diet groups. Pigs receiving diet A had the smallest decrease (1.05). The decrease was highest in diet B group (1.35).

The MCH for all diets groups reduced during the period from day 0 to 21. The rediction was lowest in pigs fed diet D (1.2) and highest in those in diet B group (1.36).

Overall, MCH decrease were 1.15, 1.36, 1.21, 1.1 and 1.25 for diet A, B, C, D and E, respectively.

The decrease in MCH is lower in pigs that consumed diet D compared to controls. Decrease in MCH is higher in pigs that received diets B, C and E. Among treatments pigs fed diet C had a lower decrease while those fed diet E had a higher decrease.

Decrease in MCH in pigs that consumed diet D was always numerically lower than the controls while pigs fed diet B remained higher than controls throughout the study period.

As can be observed from Annex 1 and Table 6, MCH changes did not vary with time (p=0.7722). Least-significant means for MCH change during the period from day 0 to 14 was -1.05 pg for the control animals. For the period from 0 to 21 these MCH change was -1.25 pg.

3.3.2.2.6 Mean corpuscular haemoglobin concentration changes

Annex 1 gives the statistical significance of effects of farm, diet, day and their interactions on mean corpuscular haemoglobin concentration mean of pigs.

Least-square means for mean corpuscular haemoglobin concentration mean changes are presented in Table 6.

MCHC changes in pigs varied over time (p=0.0065). LSMeans for MCHC levels were not different for piglets fed the control or the probiotic-supplemented diets (p = 0.6814). Interactions of farm x diet, farm x diet x day, diet x day were not detected and farm had no significant effect on MCHC of pigs (p>0.05) (Annex 1).

Overall, Trial means for MCHC reductions were 0.75, 4.99, 1.14, 0.99 and 0.25 for diet A, B, C, D and E, respectively.

As can be observed from in Table 6, the levels of MCHC varied with time (p=0.0065) and they increased in pigs that consumed diets A, C, D and E from day 0 and peaked on day 14 and then reduced with a higher margin from day 14 to 21 to reach a levels lower than at day 0. However, MCHC in pigs that received diet B decreased by a small margin (0.62) from day 0 to 14 compared to a reduction 9.36 from day 0 to 21.

The differences between the decrease/increase in MCHC in pigs between day 0 and 21 (d21-d0) and between day 0 to 14 (d14-d0) was significant and was higher between d21-0 than d14-0 (p<0.05).

As can be observed from Annex 1 and Table 6, MCHC changes varied with time (p=0.0065). Least-significant means for MCHC change during the period from day 0 to 14 was 2.54 g/L for the control animals. For the period from 0 to 21 these MCHC change was -4.05 g/L.

3.3.2.2.7 Corpuscular haemoglobin concentration mean changes

Statistical significance of effects of farm, diet, day and their interactions on CHCM changes are presented in Annex 1.

Means for effects of probiotic-supplemented diets on corpuscular haemoglobin concentration and changes in weanling pigs are given in Table 6.

CHCM changes for pigs varied over time ($p<0.0001$) and they increased in all diet groups from day 0 to 14 and reduced by a higher margin from day 14 to 21. Farm had an influence on CHCM levels of pigs ($p<0.0001$) [data not shown]. An interaction of farm x day ($p=0.0351$) was evident [data not shown]. LSMeans for CHCM levels were not different for piglets fed the control or the probiotic-supplemented diets ($p = 0.8469$) and interactions of farm x diet, farm x diet x day and diet x day ($p>0.05$) were not detected.

Overall, least-square means of CHCM increased / decrease were +0.49, -0.67, +0.23, +2.27 and +1.75 for diet A, C, D and E respectively

From day 0 to 14 (d14 - d0), CHCM increased in all diet groups. Pigs receiving diet D had the highest increase (7.01) and lowest in pigs fed diet B (3.81).

The LSmeans of CHCM for all diets groups reduced during the period from day 0 to 21. The reduction was lowest in pigs on diet E (2.13) and highest in pigs in diet B group (5.16).

As can be observed from in Annex 1, the levels of CHCM varied with time ($p< 0.0001$) and they increased for pigs in all diet groups from day 0 and peaked on day 14 and then reduced with a higher margin from day 14 to 21 to reach a levels lower than at day 0.

Pigs fed diet C had numerically lower CHCM levels on day 0 and the remained low throughout the study period.

As can be observed from Table 6 and Annex 1, CHCM changes varied over time ($p<0.0001$). Least-significant means for WBC changes during the period from day 0 to 14 was 4.73 g/L for the control animals. For the period from 0 to 21 these CHCM changes were -3.74 g/ L.

3.3.2.2.8 Red cell distribution width changes

Statistical significance of effects of farm, diet, day and their interactions on RDW changes are presented in Annex 1.

Least square means for effects of probiotic-supplemented diets on corpuscular haemoglobin concentration mean changes in weanling pigs are presented in Table 6.

LSMeans for RDW levels were not significant different for piglets fed the control or the probiotic-supplemented diets ($p = 0.2359$). RDW changes varied over time ($p=0.0432$) and the decline was greatest during week 2. Farm had an influence on RDW changes ($p<0.0001$). Interactions of farm x diet, farm x day, farm x diet x day and diet x day ($p>0.05$) were not detected.

From the start of the experiment to the end of week 2 (d0 to d14) least-square means of RDW decrease/increase were -0.1, +0.7, -0.35, -0.82 and -0.22 for diet A, B, C, D and E, respectively. The increase for the control diet was 800% lower, 250% and 720% lower, and 120% higher than diet B, C, D and E, respectively.
The decrease was higher in diet C, D and E than contrl while B had and increase.

From the start of the experiment to the end of week 3 (d0 to d21) least-square means of RDW changes (decrease) were -0.55, -0.29, -1.5, -1.71 and -1.01for diet A, B, C, D and E respectively. The decrease in RDW was higher in pigs fed diets C, D and E than control while B was lower than control anmals.

Overall least-square means of RDW decreased by -0.32, +0.2, -0.93, -1.27 and -0.062 for diet A, C, D and E respectively but increased by 0.2 for diet B.
As can be observed from in Annex 1, the levels of RDW varied with time (p=0.0432) and they decreased in pigs that consumed diets A, C, D and E from day 0 and reduced further from day 14 to 21 to reach levels lower than at day 0. However, pigs that received diet B had an increase in RDW from day 0 to 14 and then reduced from day 14 to day 21.

The differences between the decrease / increase in RDW pigs between day 0 and 21 (d21-d0) and between day 0 to 14 (d14-d0) was significant ($p<0.05$). RDW decreased in pigs that consumed diet A, C, D and E from day 0 to 14 (d0-14) and further decreased by a higher margin from day 0 to 21 (do-d21) ($p<0.05$). Pigs fed diet B had an increase in RDW between d0-d14 and a reduction from d0-d21.

As can be observed from Table 6 and Annex 1, RDW changes varied with time (p=0.0432). Least-significant means for RDW change during the period from day 0 to 14 was -0.1% for the control animals. For the period from 0 to 21 these RDW change was -0.55%.

3.4 Effect of probiotic- and lactoferrin-supplemented diets on Lymphocyte to neutrophil ratio (stress factor)

Forthly, we then investigated the effect of dietary treatment on lymphocyte to neutrophil ratio (Stress factor) of pigs. Blood was collected on weaning day, day 14 and 21 of the study period.Lymphocyte to neutrophil ratio were determined by dividing the lymphocyte by the neutrophil cell numbers of each pig at samplimg time. Lymphocyte to neutrophil ratio is a crude indicator of stress).

Annex 1 shows the statistical significance of effects of farm, diet, day and their interactions on lymphocyte to neutrophil ratio of pigs.

Least square means for effects of probiotic-supplemented diets on lymphocyte to netrophil ratio in weanling pigs are shown in Table 7.

Table 7: Effects of probiotic- and lactoferrin-supplementation on lymphocyte to neutrophil ratio (stress factor) in weaned piglets

Parameter		A (control)	B (probiotic B)	C (probiotic B)	D (probiotic B)	E (lactoferrin)	SE
Effect on *lymphocyte to neutrophil ratio*	day 0	0.8151	1.103	0.982	1.246	1.069	0.08
	day 14	1.0657	1.011	1.041	1.422	1.114	0.08
	day 21	0.8655	0.878	0.87	1.318	0.898	0.08
	difference						
	day14-day0	0.2506	-0.09	0.059	0.176	0.044	
	day21-day0	0.0504	-0.23	-0.11	0.072	-0.17	
	Trial mean	0.9154	0.997	0.964	1.329	1.0271	0.05

SE standard error

Statistical significance of effects of farm, diet, day and their interactions on lymphocyte to neutrophil ratio are presented in Annex 1. An interaction of farm x diet ($p=0.0168$) was evident while interactions of farm x day and farm x diet x day and diet x day were not detected ($p>0.05$). Farm had a significant effect on lymphocyte to neutrophil ratio of pigs ($p=0.0013$).

Least-square means for lymphocyte to neutrophil ratio was not different for piglets fed the control or the probiotic–supplemented diets B, C, D and E ($p = 0.1076$). SF were not affected by time ($p>0.05$).

On weaning day, the least-square means of lymphocyte to neutrophil ratio was 1.06657, 1.0113, 1.041, 1.422 and 1.1137 for diet A, B, C, D and E, respectively. All diet groups had an increase in lymphocyte to neurophil ratio from weaning day to day 14, except diet D group that reduced from 1.1029 to 1.013. Pigs that consumed treated diets had higher SF compared to the control animals. Among treated diets pigs fed diet D had a higher SF and was lower in pigs fed diet C

In the last week (week 3) the least-square means of lymphocyte to neutrophil ratio were 0.8655, 0.878, 0.8703, 1.3182 and 0.8981 for diet A, B, C, D and E, respectively. From day 0 to day 21 all diet groups had a reduction in SF.

On day 14, pigs fed diet B and C had lower SF than the control while SF were higher in pigs fed diets D and E than the controls.

Overall LSmeans of lymphocyte to neutrophil ratio for piglets fed the control and probiotic-supplemented diets for the 21 d period were 0.9154, 0.9974, 0.9644, 1.3289 and 1.0271 for diet A, B, C, D and E, respectively. Lymphocyte to neutrophil ratio was higher in the treated groups compared to the control. Among treated diets SF was higher in pig that consumed diet D and lower in those that received diet.

Although lymphocyte to neutrophil ratio did not vary with time ($p>0.05$), in pigs fed the control diet, lymphocyte to neutrophil ratio increased from 0.82 at day 0 (28 days) to 1.07 at day 14 (42 days). From day 14 (42 day), lymphocyte to neutrophil ratio declined 0.87 at day 21 (49 days old) (Table 6).

On day 7, pigs on probiotic- and lactoferrin supplemented diets had higher SF compared to the controls. Among the treated groups, pigs fed diet D had a higher SF and was lowest in pigs that received diet C. On day 14, pigs fed diets B and C had lower SF than the controls while SF was higher in pigs fed diets D and E than the controls. Among treated diets, pigs that consumed diet C had lower SF values than pigs in diet D. On day 21, pigs in probiotic-added diets had higher SF than the control animals. Among the treated groups, pigs that consumed diet D had higher SF while those fed diet C had lower SF. Diet D stimulated the highest stress factor of all the five diets followed by diet E and B while diets C and A caused the least effective.

Although SF in pigs were not affected by time ($p>0.05$), in pigs fed the control diet, SF increased from 0.8151 at day 7 (35 days) to 1.0657 at day 14 (42 days). From day 14 (42 day), SF increased to 0.8655 at day 21 (49 days old) (Table 6).

3.5 Effect of probiotic on Mean Weekly Faecal Scores

Fifthly, we investigated the effect of dietary treatment on mean weekly faecal score (MWFS) as described earlier (See Materials and Methods, p. 28).

Annex 1 shows the statistical significance of effects of farm, diet, day and their interactions on faecal score of pigs.

Least square means for effects of probiotic-supplemented diets on mean weekly faecal score in weanling pigs are summarized in Table 8.

Table 8: Effects of probiotic- and lactoferrin-supplementation on mean weekly faecal scores in weaned piglets

Parameter		A (control)	B (probiotic B)	C (probiotic B)	D (probiotic B)	E (lactoferrin)	SE
mean weekly faecal score	day 14	2.8067	3.221	3.238	2.869	3.106	0.14
	day 21	3.0033	3.156	3.237	2.956	3.125	0.14
	difference						
	d14-d7	-0.662	0.082	-0.43	-0.12	-0.01	
	d21-d7	-0.466	0.016	-0.43	-0.03	0.007	
	Trial mean	3.0929	3.172	3.38	2.936	3.117	0.08

SE standard error

Least-sigificant means for weekly fecal scores was not different for piglets fed the control or the probiotic–supplemented diets B, C, D and E ($p = 0.1260$). Effects of farm and day on MWFS were not observed ($p > 0.05$). Interactions of farm x diet, farm x day, farm x diet x day and diet x day were not detected ($p>0.05$).

In week 1, the least-square means of MWFS were 3.4688, 3.1396, 3.6635, 2.9844 and 3.117 for diet A, B, C, D and E respectively.

In week 2 the least-square means of MWFS were 2.8067, 3.2214, 3.2384, 2.8686 and 3.1062 for diet A, B, C, D and E, respectively.

In the last week (week 3) the least-square means of MWFS were 3.0033, 3.156, 3.2365, 2.9557 and 3.12471 for diet A, B, C, D and E, respectively without significant differences.

The MWFS were higher in diet B, C and E than control and lower in diet D group.

Overall LSmean for MWFS for the 21 d period were 3.0929, 3.1723, 3.3795, 2.9362 and 3.1165 for pigs receiving probiotic/diet A, B, C, D and E, respectively.

Although MWFS in pigs were not affected by time ($p>0.05$), in pigs fed the control diet, MWFS decreased from 3.4688 at day 7 (35 days) to 2.8067 at day 14 (42 days). From day 14 (42 day), MWFS increased to 3.0033 at day 21 (49 days old) (Annex 1). MWFS was numerically lower in pigs that consumed diets D and E compared to the control and higher in those that received diets B and C compared to the contrls.

Some pens with higher MWFS also had wet floors indicating some self cleaning mechanisms which is part of physical barrier defense mechanism.

3.6 Effect of probiotic- and lactoferrin-supplemented diets on Health parameters

There were no significant differences due to treatment observed in cumulative diarrhoea cases, other diseases, cull rate and survival rate from 0 to day 21 of the experimental period. Control animals as wellas piglets fed probiotic-supplemented diets appearered clinically normal during the whole experiment and no mortality resulted from consuming probiotics. No signs of toxicity were observed in all groups.

Piglet mortality was nil in all diet groups. Piglet mortalities after weaning were not positively influenced in the probiotic group. In fact, the piglets that were euthanased were from the pigs that received diets supplemented by probiotic B and D. In our study the probiotic supplemntation did not improve piglet losses.

CHAPTER 4

4. DISCUSSION

Effect of mixing piglets from different farms

The data showed a strong farm effect, that is mixing of piglets at the start of the trial was significant ($p<0.05$). As possible explanation for this, it can be hypothesized that different hygienic status i.e. different pathogen loads present in different housing and management systems are different from farm to farm and might affect animals responses to different parameters investigated. For example animals' responses to β-glucan by Decuypere *et al.* (1998) using animals from two different farms were different due to hygienic status. ADG was higher in pigs from low hygienic status. Eurell *et al.* (1992) reported high haptoglobin serum concentrations which are in general elevated during immune challenges and under poor hygienic conditions in pigs. Pigs may also come from areas with some remaining colostral antibodies or earlier individual antigen contact (Hiss and Sauerwein, 2003). It has also been found that piglets from different farms may have diffent number of WBC. This might be as a result of different management. For example WBC will show a slower increase if pigs are given one iron injection compared to those given two injections (Johansson *et al.*, 2005).

Effects of probiotic- and lactoferrin supplementation on physical performance and immunity of weaned pigs

To study the effects of probiotics and lactoferrin on the performance and immunity levels of weaned pigs, we mixed different breeds of pigs from four different farms to stimulate the immune challenge. This immune challenge model may be considered satisfactory but it would have been better to use a more potent immunological challenge. Stress has been implicated as a factor that stimulates the infection and (in case of swine dysentery) can also be triggered deliberately by the introduction of corticosteroid drugs or starvation of pigs (van Heugten *et al.*, 1994a). Although pigs that consumed diets D and E had significantly lowered feed intake, and those that received diet B ate significantly higher amounts than the controls, the body weights at different weighing dates were not significantly reduced by the low intake that resulted

from this immunological insult. There were no significant differences in iron levels in pigs on weaning day. Diets could have had another impact on trying to affect iron levels and iron levels were numerically higher in pigs fed diet A, B and C compared to those fed diet D and E. This agreed with the findings of Mahan and Lepine (1991) who reported that factors such as age, body weight, stress, health status, low feed intake, diet composition, digestive incompetence, and environment at weaning are the main factors of growth check.

The fact that no adverse side-effects were observed, the five diets could be tentatively be classified as safe. Good probioticts and other natural alternatives should be safe for inclusion in animal feeds and have both growth promoting effects and GRAS status (Fuller, 1992; Saxellin *et al.*, 1999; Bernardeau *et al.*, 2002). The probiotics and lactoferrin used in the trial were non-pathogenic and safe for animal consumption in addition probiotics B and C had a beneficial effect on growth performance (feed intake) in weaned piglets. Further experiments are, however, warranted.

Comparatively, depression of body weight in infected animals was higher [about 1000g (8.5kg in health compared to 7.5kg in sick pigs)] in van Heugten *et al.*,'s (1994a) study and was only slightly lowered [by only 200g (8.5kg in healthy (van Heugten *et al.)* pigs – 8.3kg in our controls) on day 7 post challenge] in our study compared to controls in published studies (van Heugten *et al.*, 1994a). The reduction in body weights in other diet groups in our study, compared to the controls in van Heugten *et al.*,'s study during the same period, was 100, (8.5kg in healthy van Heugten *et al.*'s pigs – 8.4 in diet B pigs), 200 (8.5-8.3 diet C), 300 (8.5-8.2) and 500 (8.5-8) for diet B, C, D and E, respectively. Similarly, the body weights were lower than in van Heugten *et al.* (1994a) because of the beneficial effect of these probiotics.

Although we did not have an unchallenged study in the current study, our results can be compared with those reported by van Heugten *et al.*(1994a). They used feed that was similar in nutritive value and pigs that had similar body weight on day 0 of the challenge (7.3kg in van Heugten *et al.* vs 7.5kg in our current study at 28 days old). However, the differences in environment and management between the experiments, which are known to affect performance, were excluded. In their study, the body weights in the controls were 7.2kg, 8.5kg, 10.8kg and 14.0kg at age 28, 35, 42 and 49

days old, respectively. In their study, ADG of controls were 190, 320 and 470 g per day at age of 35, 42 and 49 days old, respectively while ADFI was 390, 630 and 900g per pig per day at age of 28, 35, 42 and 49 days old, respectively. FCR of controls were 2.0408, 1.9608 and 1.9231 at age of 28, 35, 42 and 49 days old, respectively. With this basic information, we can see that in our study the body weights of pigs were depressed by the immune challenge caused by mixing piglets from different farm sources. They had numerically lower iron levels and the body weights in the control group were lowered by 200g, 600g and 100g at age of 35, 42 and 49 days old, respectively, compared with unchallenged pigs in van Heugten *et al.*,'s experiment. In our study ADG, ADFI and body weights were all reduced by weaning and the immune challenge compared with the result if animals were not challenged.

Reduced feed intake is a factor that limits growth in weaned pigs. Weight is gained after the improvement in feed intake (Davies *et al.*, 2002).The depression in body weight was high in week 1 and even higher in week 2 but the FI, ADG and body weights increased in pigs fed diet B and C (14.057kg and 14.005kg bodyweight for diet B and C, respectively) to outperform the unchallenged pigs (14.0kg) in van Heugten *et al.*,'s (1994a) study on day 21 post challenge. However, those fed diet A, D and E performed poorer compared to controls in van Heugten et al.,'s study (13.866kg, 13.481kg and 13221kg body weight for diet A, D and E, respectively). The depression in ADG, FI and body weights in our experiment agrees with those of others (van Heughten *et al.*, 1994a, 1994b; Kegley *et al.*, 2001; Barnett *et al.*, 1989; Pluske *et al.*, 1997) and indicates that after weaning / immunological challenge or lower iron levels, animals reduce either ADG, FI, FCR and bodyweight. Animals also attempt to develop a tolerance to the immune challenge and those fed diet B and C in our study mounted a greater immune response by day 21 than pigs fed diets A, D and E. On the other hand, animals continuously flooded with a polysaccharide antigen may prevent development of an immune response and pigs will quickly surrender to bacterial infection when put under stress or immunological challenge. This is referred to as immunoparalysis and takes place when excess antigen is congested by circulating antibodies as they are formed (Barnett, 1983).

Furthermore, Marin *et al.* (2002) reported reduced feed intake and body weights in pigs that received 140 and 280 ppb aflatoxin intoxicated feeds compared to healthy

controls (0 ppb aflatoxin). The body weights and body weight depression reported by Marin *et al.* were greater than those reported in our study or by van Heugten *et al.* (1994a). This work found bodyweight differences between the probiotic-supplemented and unsupplemented controls and also among supplemented pigs themselves.

Differences in body weights of pigs fed diet D and E and controls increased with time. This work concurs with other experiments; weight differences between pigs that consumed 0 ppb or 280 ppb aflatoxin diets (Marin *et al.*, 2002) and also between the unchallenged and infected pigs in van Heugten *et al.* (1994a), increased with time as the feeds did not contain any treatments to support immunity against prolonged high levels of the intoxicating agents (immunoparalysis). Svoboda *et al.* (2004), also reported decreased body weight in anaemic animals compared to those with adequate HGB in early stages of life. It is interesting to note that after the immune challenge in van Heugten *et al.* (1994a) and Marin *et al.* (2002), body weight after the immunological challenge remained lower in challenged pigs compared to the controls for the whole duration of these studies, although feed intake was depressed only for a short time immediately after the challenge but improved to outperform the control (in van Heugten *et al.*,'s (1994a). It is also interesting to note that animals with less stress (140 ppm aflatoxin and those challenged only once) tried to compensate FI and ADG and body weight (tolerance) while those with higher and continuous stress (of 280 ppb aflatoxin and those challenged for second time with lipopolysaccharide) failed to compensate body weight even though they tried to compensate for FI and ADG (immunoparalysis).

In our study, pigs that received diet D and E had significantly lower feed intake, lower ADG ($p>0.05$) and had lower body weights ($p>0.05$) for the entire period of the experiment compared with those fed control diet, diet B and C. With these findings we can conclude that probiotics B and C offered a rapid and better immune response, thus an improvement in feed intake which also compensated for higher body weight to outperform the controls. In contrast, pigs that received diets D and E failed to compensate for ADG, FI and body weight due to reduced immunity although they had numerically a higher or more efficient FCR ($p>0.05$) than pigs fed diet B and C. Lower feed intake might have been worsened by the numerically lower iron levels

(low HCT, HGB and RBC). It is interesting to note from van Heugten *et al.*, (1994a, 1994b) and Marin *et al.*,'s (2002) studies that improved feed intake in immunoparalysed animals does not automatically lead to higher compensatory body weight gain with healthy animals. Reduced ADG, FI, FCR and body weight in pigs have also been documented in many other studies (Klasing *et al.*, 1987; van Heugten *et al.*, 1994; Owusu-Asiedu *et al.*, 2002; Coma *et al*, 1995; Jonasson, 2004; Spurlock *et al.*, 1997; Marin *et al.*, 2002; Odink *et al.*, 1990a; Svoboda *et al.*, 2004) caused by change in diet or stress.

Important lessons from van Heugten *et al.* (1994a) are that reduced FI automatically results in body weight reduction and even when FI is quickly restored or significantly increased in challenged pigs to outperform healthy controls, bodyweight is not automatically restored or improved to reach that of healthy pigs. In addition, even though ADG was only slightly (and not significantly) reduced in pigs fed 140 ppb aflatoxin compared with controls in Marin *et al.*'s (2002) study, body weight was significantly reduced by a wider margin in immunologically insulted pigs compared to controls. Therefore, performance data should be discussed because even those data that are not statistically significant (e.g. FI and ADG) can significantly affect other performance parameters (e.g. body weight or body weight gain).

Interestingly, different investigations have reported contradictory results when natural products such as garlic are used. Grela *et al.* (1998) reported an increase in feed intake when garlic was used in pigs while Corrigan *et al.* (2001) found that garlic in the diet of nursery pigs decreased feed intake. However, garlic used by Corrigan *et al.* (2001) was used in combination with a mixture of plant extracts, mixed herb and essential oils. Therefore, it is likely that the other herbs and plant extracts may have hidden the strong odour of garlic. This indicates that it is the organoleptic properties of garlic that are to blame for the decrease in feed intake in pigs (Cullen *et al.*, 2005). Similarly, in our study, palatability of diet D and E may have been lower than that of diet B and C as pigs preferred the last two diets. As the evidence presented above indicates, the sense of taste is involved in controlling the selection of food by pigs (Baldwin, 1976) while the influence of olfaction is also recognised (Forbes, 1995). In pigs the sense of smell is highly involved in feed intake (Mellor, 2000). The active ingredient in garlic

(allicin) is a remarkably odoriferous compound (Cavalito & Bailey, 1994). It can be seen in the investigation by Cavalito and Bailey, 1994, that the decrease in feed intake was not significant during the finisher period, suggesting that animals may quickly get used to garlic (Cullen *et al.*, 2005). Similarly, in our study, the initial reduction in FI in all pigs post challenge and the increase in FI in pigs fed diets B and C, could be due to this reason.

Feed intake is a factor limiting growth in weanling pigs. Weight gain accompanies the improvement in feed ingestion. Probiotic may act on the gut to improve performance as observed by an increase in feed intake (Davies *et al.*, 2002). Ingestion of probiotic D and E in our study, or for lipopolysaccharide in van Heugten *et al.*, 's (1994a) study, reduced FI and body weight. However, it is very surprising to note that despite a reduction in feed intake (and digestible energy associated with garlic inclusion in Cavalito & Bailey's (1994) experiment), there was no negative effect of garlic on liveweight gain. The inclusion of garlic in the diet at 1 g/kg improved the feed conversion ratio by 9.8% during the grower period and 5.7% during the combined grower finisher period, while the inclusion of garlic at 10 g/kg improved FCR by 7.0% during the grower period and 6.5% during the combined grower-finisher period, when compared to the control diet (Cullen *et al.*, 2005). The improvement in FCR reported above by Cullen *et al.* (2005), using garlic, and the improvement of both FCR and daily gain reported by Grela *et al.* (1998), using great nettle, garlic and wheat grass mixture, were all much greater than the improvement in FCR or gain noted in studies using antibiotics as growth promoters (Zimmerman, 1986).

Some probiotics have the ability to influence immune response (Bloksma *et al.*, 1979). Some beneficial effects of direct-fed-microbials are that they influence immunoadjuvant activity (Perdigon *et al.*, 1991) and increase the total amount of intestinal IgA (Tizard, 2000; Lin, 2000). In contrast, Kluber *et al.* (1985), working with weanling pigs, observed no effect of probiotic on the cell-mediated immune response.

The improvement in feed efficiency by pronutrients / phytogenics such as garlic, might occur for example via (1) the improvement of gut environment and microbial

flora. The explanation for this is traced to the fact that the susceptibility of harmful gram positive bacteria to the antibacterial compounds in garlic is higher than that of the beneficial bacteria in the gut (Rees *et al.*, 1993). The desirable bacteria are said to be protected by the presence of garlic as they are less sensitive to its inhibitory effects. Furthermore, garlic containing fluctooligosaccharides, may have a prebiotic effect on gut microflora (Gibson, 2001); (2) the improvement in feed efficiency with the inclusion of garlic may be blamed on the lower DE intake of pigs offered the garlic diet compared to those offered the control diet. In finishing pigs, FCR improves with increasing energy intake up to 33 MJ DE/day but becomes less efficient with each increase in DE intake thereafter (Campbell *et al.,* 1985 (cited in Cullen *et al.*, 2005)). Similar trends in FCR were reported by O'Doherty and McKeon (2000) when using similar genotypes as in Cullen *et al.*,'s (2005) experiment and (3) antimicrobial action of allicin (Ankri & Mirelman, 1999) may have the ability to prevent microbial fermentation in the gut. In addition garlic has antiviral activity (Ankri & Mirelman, 1999).

The gut and the skeletal musculature in fast growing pigs is derived from a limited supply of nutrients and are, in effect, antagonists for the accretion of nutrients (Rees *et al.*, 1993). Vervaeke *et al.* (1979) reported that up to 6% of the net energy in pig rations cannot be used for the benefit of the pig due to bacterial utilisation of glucose in the small intestine. The requirement of amino acids in these bacteria is similar in amount to that of growing pigs (Hays, 1978). Inclusion of garlic in feed at 10 g/kg might stimulate a nutrient sparing and / or economising effect, hence improving FCR (Cullen *et al.*, 2005). Some workers have reported that garlic improved performance (Cullen *et al.*, 2005; Ankri & Mirelman, 1999; Janz *et al.*, 2007; Horton *et al.*, 1991) while others have reported no effect (Reddy *et al.*, 1998). These inconsistencies in results have been found in many probiotics and in garlic, and could be due to variable inclusion levels of garlic and in the allicin and alliin concentrations of the garlic used (Cullen *et al.*, 2005).

Several possibilities for why natural products such as probiotics have not been consistently successful include: insufficient bacterial cell numbers; bacteria incapable of surviving and performing their metabolic functions in the gut, which may be influenced the presence of antibiotics in the feed (Turner *et al.*, 2001); ageing bacteria

cultures losing their efficacy over time; and instability of intestinal fermentation (Hillman, 1999). The latter can be as a result of overdosing with the probiotic organism such that it exhausts all available nutrients and diminishes other beneficial bacteria as well as pathogenic bacteria. In some cases, the probiotic may replace a colony of harmful bacteria while in others, the probiotic may replace a colony of beneficial bacteria, thus cancelling out any benefit it may have provided (Ewing & Cole, 1994). In other studies, it has been seen that some probiotic bacteria, such as *Bacillus* spp, are not normal components of the indigenous intestinal microflora, so that those bacteria are hard to establish in the digestive tract, hence no beneficial effect (Jonsson & Conway, 1992). The use of different methods as a model for in vivo pig inflammatory conditions to investigate immune challenge such as live bacteria (for example enterotoxic *Escherichia coli- ETEC*) (Owusu-Asiedu *et al.*, 2005; Touchette *et al.*, 2000: Bosi *et al.*, 2002) or lipopolysaccharide (LPS) (van Heugten *et al.*, 1994a) can yield different results. Nutritional status, diet and low feed intake were the main problems in reduction of performance in our study.

When food components, such as lactoferrin, are naturally injested, they interact with a number of lymphoid cells as they move down the gut. Numerous factors can affect these interactions such as solid or aqueous form of the food component and the mode of exposure (Sfeir *et al.*, 2004; Fugh-Berman, 2000; Chavez *et al.*, 2006). In rats, administration of lactoferrin through injection resulted in higher stimulation of innate and adaptive immune response compare administration through drinking water (Sfeir *et al.*, 2004).

In summary, we can conclude that during the 21 day period, the effects of probiotic-supplementation on ADG, FCR and body weight were not different among diet groups ($p>0.05$). By growing fast, pigs fed diets A, B, and C would reach target weight earlier. This can reduce occupation rate thus making housing more efficient, so that more space and time is available for more pigs to be kept (Janz *et al.*, 2007). Low feed intake post weaning / post challenge (or during the time with lower iron levels,) reduced gain resulting in nutritional stress and possibly caused reduced immune resistance and obstruction of antibody formation (Barnett *et al.*, 1989). When feed intake is improved after weaning / post challenge, mucin production in the gut improves (Lopez-Pedrosa *et al.*, 1998; Lalles *et al.*, 2004). Mucin contributes to the

healthy gut through lubrication, physico-chemical protection and prevention of bacterial adhesion (Forstner & Forstner, 1994; Lalles *et al*., 2004; Tizard, 2000) acting as a self cleaning barrier mechanism. This also increases the enzymes necessary for the breakdown of starch, carbohydrares and protein (Lombardi *et al*, 2005).

During the digestion process, some dietary proteins or polypeptides may escape the luminal hydrolytic process and find their way into the intestinal mucosa in sufficient quantities, where they are, at a later stage, absorbed or incorporated through different mechanism, including both the paracellular and transcellular pathways (Tome & Debbabi, 1998). This absorption of large molecules in antigenic and biologically active amounts is presumed to activate different physiological and immunological reactions that lead to oral tolerance and its control (Bahna, 1985). Proteins, including lactoferrin, are known to interact with either minerals, vitamins or nutrients by specific mechanisms. These interactions affect incorporation or absorption of these nutrients. Casein phosphopeptides have been found to inhibit the precipitation of calcium phosphate and speed up its absorption in the small intestine (Lee *et al*., 1980).

Lactoferrin is believed to have a part to play in DNA synthesis, proliferation, differentiation and metabolic effects (Tome & Debbabi, 1998) and is involved in host defence through its bacterial activity (Lonnerdal & Iyer, 1995). Probiotic B and C might protect proteins and facilitate this system of absorption in pigs, as evidenced by a numerically higher gain and protection from immune challenge, although this was not the case for pigs fed lactoferrin-supplemented diet. Different proteins, including lactoferrin, vitamin B_{12}, protein, folate binding protein, β-lactoglobulin and α-lactalbumin are assumed to interact with either mineral, vitamin or nutrient s by a specific (Iyer & Lonnerdal, 1993; Tome & Debbabi, 1998).

The manner in which enzootic diseases affect animals is not well understood but more investigations continue to be carried out. Pathogens trigger an immune response specially designed to remove them from the body. In reaction, the immune system activation stimulates a surge of effects, some of which are beneficial to growth, on the animal's metabolic system. The type of antigen determines the clinical signs observed in the exposed animal. A non-pathogenic bacteria, a vaccine and / or a natural product may have relatively little or no harmful effects on the animal, whereas exposure to a

pathogenic antigen could severely affect the animal's performance (Williams *et al.*, 1997). We found reduced weight gain in probiotic D- and E-supplemented pigs and this was as a result of reduced feed intake. Similarly, Williams *et al.*, 1997 compared pigs from 6 to 27 kg that differed only in immune system activation and reported significant increases in growth rate and feed efficiency, and only a tendency towards increased feed intake. In addition, van Heugten *et al.* (1994a), and Pluske *et al.* (1997) all reported that an antigenic challenge in young pigs suppressed the growth rate, with its greatest impact on feed intake rather than feed efficiency.

Metabolic changes linked to infectious diseases can result in decrease in gain and feed efficiency. The following studies by Klasing *et al.* (1987), van Heugten *et al.* (1994a), (1994b), Coma *et al.* (1995), Jonasson (2004), Spurlock *et al.* (1997), Marin *et al.* (2002), Odink *et al.* (1990b) and Svoboda *et al.* (2004) found decreased weight gain and / or feed intake and / or efficiency of feed utilisation in animals that were repeatedly challenged with noninfections or infectious (immunological) agents or other stress. The metabolic switch following immune challenge are caused by interleukin 1 (IL 1) and tumor necrosis factor (TNF) produced by stimulatory macrophages (Morrow-Tesch & Anderson, 1994; Marin *et al.*, 2002; Klasing, 1988; Hiss & Sauerwein, 2003). Nutrients are prevented from reaching intended growing sites (increase in size and volume of cells) and are redirected to the mobilisation of a defence system (Demas *et al.*, 1997; Hiss & Sauerwein, 2003; Beisel, 1977; van Heugten *et al.* 1994a, 1994b and 1996; Owusu-Asiedu *et al.* 2003; Spurlock, 1997; Davis *et al.*, 2002). The amount of nutrients being used for the immune system were quickly reversed in pigs that received diets (A,) B and C and this is evidenced by beneficial effects on growth performance originating from increased ADFI which improves ADG. This is consistent with Hiss and Sauerwein (2003) who observed reduced immune function in weaned / challenged pigs that consumed higher amounts of feed supplemented with β-glucan compared to the controls and van Heugten *et al.* (1994a) who reported reduced weight in lipopolysaccharide challenged piglets. In addition, lowering of interleukins and tumor necrosis factors, which are inhibitors of feed intake, by probiotics, mediated by the central nervous system, might contribute to the increased feed intake (Hiss & Sauerwein, 2003). Similarly, increase in feed intake in our study could be due to increases as a result of the removal of these inhibitors in pigs fed diet B and C, compared to those fed diet D and E.

In our study, feed intake was a factor limiting growth in young pigs and daily gain and body weight was, therefore, improved with increasing intake (in pigs fed diets B and C). This is consistent with published research of Davis and coworkers (2002) and Janz *et al.* 2007 who reported an increase in gain and feed intake when $CuSO_4$ and garlic, respectively were added to feed. Increased feed intake that occurs when natural products such as MOS or copper are added is as a result of reduction of intestinal damage caused by pathogens because of their antimicrobial action. Systematic administration of certain minerals such as copper can improve many functions that they serve in the body.

The significance of diet on the immune system was difficult to detect. The explanation could be that piglets were only challenged once or lightly immunologically challenged and pigs consumed 'adequate' weaning rations with a high protein and energy content. This concurs with Lopez (2000) who reported that in feeding a well formulated diet and / or under good environment, there is no opportunity to further improve nutrients digestibility and the performance of the piglets.

The integrity of the gut mucosa is a prerequisite to lower the entry of pathogens (Bosi, 2000). The components of the gut mucosal barrier (non immunological and immunological) are, however, disturbed during stress such as at weaning. Lowered feed ingestion in the weaned pig causes villous to reduce in size (Bosi, 2000). A lowered supply of milk (Kelly *et al.,* 1991) or a restricted ingestion of dry feed (Pluske *et al.,* 1996) reduce the height of villous on the fifth day post weaning. Inadequate feed intake in weaned piglets may result into intestinal inflammation and affect intestinal morphology. In addition, the antigenicity of the diet is another factor (Bosi, 2000). The defense against pathogens is integrated by the endogenous secretion of many antimicrobial components such as hydrochloric acid, lactoferrins, mucous secretion. Some of these, however, the mechanisms of control of secretion are not adequately known to increase their production by dietary means (Bosi, 2000).

In our study, piglets did not respond well to the lactoferrin treatment as they were in good hygienic conditions, which would probably result in decreased opportunity for

lactoferrin activity. This result is also consistent with those of other workers. Sarica *et al.* (2005) reported that well nourished healthy chicks do not positively respond to growth-promoters when they are housed under clean conditions and at a moderate stocking density.

Future research should be conducted on commercial farms and more potent immune stimulants should be used continuously or at several times during the study.

Good feeding or good nutrition involves formulating a feed to meet the requirements of an animal and can therefore stimulate production and improve health. Some nutrients / elements or natural products such as probiotics have the characteristic concentrations and functional forms that should be maintained within narrow limits to maintain a functional and structural integrity of the tissue to safeguard growth and health. Nutrients such as iron (measured as haemoglobin) are important components of tissue (such as blood) and blood components (such as transferrin and ferritin, erythrocytes, leukocytes and monocytes) which play an important role in maintenance of health and a deficiency / excess may result in ill health (Underwood & Somers, 1969; Underwood & Mertz, 1987).

Except for ADFI, other parameters studied (ADG, FCR, blood parameters, mean weekly fecal scores (MWFS) and general health) were not significantly different between diet groups, although some numerical diffences were observed.

CHAPTER 5

5. CONCLUSION

The present work has researched development of treatments B, C, D and E as alternatives to antibiotics for improving physical performance, general health and immunity in weaned pigs. We have assessed the five diets (A(control), probiotic B, C, D and diet E (lactoferrin)) for their effects on average daily gain, feed conversion, feed intake, mean weekly fecal scores (MWFS), blood parameters, stress factors, and general health. These are essential parameters that can be explored and utilised to find effective and safe natural products to be used to reduce diseases and improve the productivity of pigs while at the same time protecting the environment.

The findings have shown that weaning a piglet from its mother's milk to a different diet inhibits growth due to hypersensitivity. Stress and some allergenic components contained in the weaned diet can cause scours and prevent growth to some extent. The intestinal microflora is usually stable but is dynamic and can be perturbed by changes in the gut environment (Kelly, 1998). Growth lag, high mortality, morbidity and diarrhoea have been reported in many studies after weaning / immune challenge (Lalles *et al.*, 2004). Restoration of the microbial balance of the intestines is the basic tenent of probiotic therapy (Kelly, 1998). Depending on the severity of stress, diet, and animal capacity, the pigs may have a reduced feed intake or feed conversion and may have reduced bodyweight. One of the factors that limit growth in weaned piglets is feed intake (Davies *et al.*, 2002). Weight is gained after the improvement in feed ingestion. Probiotic B may have a beneficial action on the gut to improve performance as observed by an increase in feed intake. As pointed out by Jensen (1998), a good diet will produce a stable gut ecosystem that has adequate capacity to resist change as micro niches in the gut ecosystem are well covered and available energy sources are quickly used for the benefit of the host. Immunocompromised animals may fail to compensate body weight even if feed intake is later improved. On the other hand an animal fed an adequate diet, may develop a tolerance and will compensate for body weight similar to or higher than if they were not stressed. Under these conditions diet B and C were "adequate" diets while D and E were not. A diet or probiotic used in

weaned pigs should be able to stimulate feed intake, FCR and / or daily gain and most importantly, compensate for body weight even when pigs are immunologically challenged. On the other hand when feed intake is reduced, a good diet should improve feed conversion and utilisation so that nutrients are better used for growth and lead to bodyweight compensation.

Supplementation of probiotic B and C improved feed intake compared to the control diet. The improvement was only significant in pigs fed diet B. Although, FCR, MWFS, SF and blood characteristics were not significant, some numerical differences and general improvement in pigs were observed between diet groups. ADG followed the pattern of feed intake and was numerically higher in pigs fed diet B and C compared to those that consumed the control diet while pigs that received diets D and E ate less feed compared to the controls. These direct-fed microbials (B and C) can, therefore, be safely used as natural growth promoters and as alternatives to antibiotics in weaned pigs.

It is important to report all findings as even non-significant results may significantly affect other performance parameters. Probiotics B, C, D and diet E (lactoferrin) stimulated feed intake performance differently ($p<0.05$), but there was no significant difference between the diet in the manner in which they affect daily gain and feed conversion performance. However they could be further developed and marketed as they can be used safely as feed additives in the place of antibiotics.

The mode of action is different and inconsistent between natural products such as probiotics, although several possibilities for why probiotics have not been consistently successful have been proposed. For example, lactoferrin is reported to reduce diarrhoea (Steijns, 2001) but the difference in mean weekly faecal scores between diet groups was not significant in our study. The failure of probiotic effect of some products may arise as a result of the environmental state such as a clean environment or a hygienic place in which, the beneficial effect may be minor (Cromwell, 2001; Yu *et al.*, 2004; Decuypere *et al.*, 1998; Hiss & Sauerwein, 2003). As earlier stated other consistencies may also be as a result of: insufficient bacterial cell numbers; bacteria incapable of surviving and performing their metabolic functions in the gut, which may be influenced the presence of antibiotics in the feed (Turner *et al.*, 2001); ageing

bacteria cultures losing their efficacy over time; and instability of intestinal fermentation (Hillman, 1999), to mention but a few. Therefore, having good nutrition, management and disease control are vital important for livestock productivity (Walton, 2001).

The performance values of controls in experiments planned to test the effect of probiotics should be low so that the biological variation between the control and treated groups are clear and therefore such studies should be conducted with respect to prevailing practical or farm conditions (Jensen, 1989; Sarica *et al.*, 2005). Effects are also dependent on diet or component, nutrients, physiological state of animal sick or pregnant, age, concentration, CFU, environment and many other factors and the effects are either localized at digestive, the systemic, or central level. All diets were safe for use in pigs. In addition, some had some benefits which resulted in similar or different effects. The diets should be used at appropriate times depending on the effect that is desired or combined to get a wider or full benefit of two or more benefits e.g. diet B improves weight better in wk 1 while diet C improves it better in wk 2. At the current concentration levels and method of preparation, (control diet or) diets supplemented with probiotic-B or C can improve FI and ADG and bodyweight and may offer some beneficial effect in gastrointestinal tract from day 0 to 21 postweaning / post challenge.

As there is only 2% of total blood that flows through the peripheral layer, this is not effective for determining diseases (Tizard, 2000; Cummings *et al.*, 2004). Blood tests cannot be relied upon for detection of immunity / health parameters. When the immune system is impaired clinical symptoms appear, although this is not always the case (Cummings *et al.*, 2004). For example, pigs can carry disease and can occasionally shed bacteria without showing clinical symptoms such as diarrhoea or differences in leukocyte population or subpopulations. Therefore, future experiments should collect blood more frequently and include post-mortem examinations to confirm any disease (Jonasson *et al.*, 2004). Haematological parameters may be used to indicate immunity but should be used together with incidences and severity of infections and growth functions (Cummings *et al.*, 2004). Haptoglobin serum concentrations are generally raised when the immune system is challenged and under poor hygiene and should therefore be included (Hiss & Sauerwein, 2003).

At weaning, scouring in piglets is mainly due to hypersensitivity. The cause of diarrhoea may vary, and the impact on health and well-being can range from slight discomfort to severe malnutrition and death in humans (Brown, 1994; Lin, 2000). After weaning scours are caused by other factors. A good diet stimulates strong chemical barriers, with high gastric acid resulting in low pH (<3.0) and this improves digestion of nutrients and stimulates iron and calcium absorption. Protein accretion for body weight gain and formation of protective components of the blood is stimulated to support health and body functions. With serious digestion stress, a good selective epithelial mucosa will trigger fast movement patterns in the bowels which is linked to elevate fluid production resulting in higher MWFS. This is an important mechanism of defense by which pathogens are flushed out of the body (self cleaning) (Boudraa *et al.*, 1990). Low MWFS is therefore not always a good indicator of gut status as animals may harbour bacteria without showing symptoms.

To sum-up, a good probiotic / diet should produce a faster and more rapid response by stimulating feed intake (feed conversion, immune challenge and growth and stimulate iron use to alleviate anaemia) in the first three weeks of life or at the times of stress such as weaning and immune challenge and enable body weight compensation. A diet should be formulated in such a way that it entirely matches the capacity of the animal's haematopoietic system for haemoglobin synthesis and growth. This should also be efficient in producing a higher immune barrier and channel nutrients for growth.

A good diet (probiotic or natural cure) should quickly improve feed intake so that pigs ingest sufficient amounts to compensate for body weight loss (such as diet B and C). Feed intake is a factor limiting growth in weanling pigs (Davies *et al.*, 2002). If feed intake (energy) is reduced, then, an effective diet should improve the feed utilisation of the available or ingested nutrients in the animal (e.g. garlic). When feed intake increases, weight gain is also improved. When this is achieved, the less nutrients consumed would be better utilised and the net energy will be sufficient to meet requirements for both maintenance and growth and most important, compensate body weight. When feed intake is improved after weaning / post challenge mucin production in the gut improves (Lopez-Pedrosa *et al.*, 1998; Lalles *et al.*, 2004;

Spreeuwenberg *et al.,* 2001). Mucin contributes to the healthy gut through lubrication, physico-chemical protection and prevention of bacterial adhesion (Forstner & Forstner, 1994; Lalles *et al.*, 2004) – self cleaning.

As feed intake increases, the levels of digestive enzymes responsible for the breakdown of starches, proteins and fats increase. Therefore making the animals to consume more feed shortly after weaning is very important (Lombardi *et al.,* 2005). As reported by Hiss and Sauerwein, 2003 and Langham and Hrupka, 1999, reduction of pathogen load by β-glucan in the systemic effect medaited by the central nervous system might contribute to increased feed intake.

A good diet should provide an environment in the gut which favours beneficial bacteria to live longer while exploitative parasites should be quickly purged / flushed out of the alimentary canal – again, self cleaning barrier mechanism. Pathogens may be reduced by using a diet (such as diet B and C) that quickly stimulates fast flow of digesta to reduce the pathogen load (Simon, 1998). As Fugh-Berman (2000) reported herd-drug interactions or probiotic-ingredient interactions are evident and probiotic reasearchers should advise about mixing probiotics and diet formulations and timing of their use.

As pointed out by Bernardeau *et al.,* 2002 and Fuller, 1992, a good probiotic should not only have growth promoting effect, but must also have a generally recognized as safe (GRAS) status.

Further studies are therefore warranted. These should also measure the number of probiotic bacteria both at the start and end of the experiments and know whether these can be retrieved live or dead. As pointed out by Bernardeau *et al.* (2002) and Fuller (1992), some bacteria can still maintain their probiotic effect whether dead or alive.

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7. LIST OF APPENDICES

Annexe 1: The statistical significance of effects of farm, diet[a], and their interactions on different parameters in weaned pigs

Parameter	farm	diet[a]	Farm x diet	day	farm x day	diet x day	farm x diet x day	R^2
Absolute cells								
WBC	*	ns	ns	***	ns	ns	ns	0.76
Neutrophils	**	ns	ns	***	ns	ns	ns	0.62
Lymphocytes	***	ns	ns	***	**	ns	ns	0.64
Monocytes	*	ns	ns	***	ns	ns	ns	0.46
Eosinophil cells	***	ns	ns	***	ns	ns	ns	0.59
Basophils	ns	ns	ns	**	*	ns	ns	0.61
RBC	***	ns	*	***	**	ns	ns	0.75
Haemoglobin	***	ns	ns	ns	*	ns	ns	0.75
Haematocrit	***	ns	ns	ns	**	ns	ns	0.74
Mean Corpuscular Volume	**	ns	ns	*	ns	ns	ns	0.81
Mean corpuscular haemoglobin	***	ns	ns	*	ns	ns	ns	0.91
CHCM	***	ns	ns	*	ns	ns	ns	0.88
RDW	***	ns	ns	ns	ns	ns	ns	0.93
Cell changes								
WBC	**	ns	ns	ns	ns	ns	ns	0.82
NEUT	*	ns	ns	ns	ns	ns	ns	0.84
LYMPH	***	ns	ns	ns	ns	ns	ns	0.84
MONO	ns	ns	ns	ns	ns	ns	ns	0.71
EOSI	***	ns	ns	ns	ns	ns	ns	0.85
BASO	***	ns	ns	ns	ns	ns	ns	0.87
RBC	***	ns	ns	ns	ns	ns	ns	0.9
HGB	***	ns	ns	ns	ns	ns	ns	0.9
HCT	***	ns	ns	ns	ns	ns	ns	0.92
MCV	ns	ns	ns	ns	ns	ns	ns	0.96

MCH	**	ns	ns	ns	ns	ns	ns	0.89
CHCM	***	ns	ns	***	*	ns	ns	0.88
RDW	***	ns	ns	*	ns	ns	ns	0.87
Mean weekly Faecal Score	ns	ns	ns	ns	ns	ns	ns	0.4
L:N or stress factor	**	ns	*	ns	ns	ns	ns	0.80

white blood cells (WBC), neutrophil cells (NEUT), lymphocyte cells (LYMPH), monocyte cells (MONO), eosinophil cells (EOS), basophil cells (BASO), red blood cells (RBC), haemoglobin concentration (HGB), and erythrocyte indices viz., haematocrit level (HCT), mean cell volume (or mean erythrocyte volume) (MCV),
mean cell haemoglobin (or mean erythrocyte haemoglobin content) (MCH), mean corpuscular haemoglobin concentration (or mean erythrocyte haemoglobin concentration) (MCHC), corpuscular haemoglobin constant (CH), Corpuscular haemoglobin concentration mean (CHCM), red blood cell distribution width (or erythrocyte distribution width)(RDW), haemoglobin distribution width (HDW), platelet (PLT) and mean packed volume (MPV); and lymphocyte to neutrophil ratio (L;N) or stress factor

[a] diet A (control), probiotic B, C, D and lactoferrin (E)
R^2 R-square

ns, *, **, *** : not significant, significant at p = 0.05, p=0.01 and p=0.001 respectively.