Growth and immunity of weaner piglets supplemented with dietary tryptophan, threonine and glutamine

by

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Declaration

I, the undersigned hereby declare that the work contained in the dissertation is my own original word and has not previously been submitted at any other university for a degree.

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Signature Date

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Abstract

Post-weaning mortality has a major economic implication for pig producers. Post-weaning stress is influenced by a number of factors, including the piglets' inherent ability and physiological mechanisms to adapt to the new environment. Various amino acids, including threonine, tryptophan and glutamine, have been shown to have a positive effect on the immune system. Higher inclusion levels than current commercial standards of threonine, tryptophan or glutamine and different combinations of these, were mixed into a basal weaner diet to create eight dietary treatments. 48 crossbred piglets (Landrace x Large White) were included in a 28 day growth trail shortly after wean, with six piglets per treatment. Body weight gain, feed intake and feed conversion efficiency (FCE) were measured weekly. At the onset of the trial, piglets were injected with a 10% ovine erythrocyte suspension as an immune challenge. Blood samples from each piglet were collected at 7 day intervals to obtain antibody titre values against ovine erythrocytes. In general, amino acid concentrations used in this study did not result in significant differences ($P>0.05$) between treatments except for a significant decrease in both body weight gain and FCE when higher concentrations of threonine were fed. However, higher antibody values against sheep erythrocytes were noted in piglets when they received additional threonine in their feed ($P>0.05$). The results of this trial were not conclusive, but indicated a negative impact of high threonine levels on the production performance of the piglets, while simultaneously improving the production of antibodies against foreign protein molecules, which could support the health of piglets during the post-weaning period.

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List of abbreviations

3HAA	3-hydroxyanthranilic acid
ADG	Average daily gain
AI	Artificially Inseminated
Ala	Alanine
ANOVA	Analysis of variance
Arg	Arginine
Asp	Aspartic acid
Asn	Asparagine
ATP	Adenosine Tri Phosphate
CO_2	Carbon dioxide
CP	Crude Protein
CSLS	Centre for the Study of Living Standards
Cys	Cysteine
DAFF	Department of Agriculture, Forestry and Fisheries
DE	Digestible energy
DM	Dry matter
DNA	Deoxyribonucleic acid
EBV	Estimated Breeding Value
FAO	Food and Agriculture Organization of the United Nations
FCE	Feed conversion efficiency
FI	Feed intake
GE	Gross energy
Gln	Glutamine
Glu	Glutamate

Gly	Glycine
HCl	Hydrochloric acid
His	Histidine
HPLC	High performance liquid chromatography
IFN-γ	Interferon gamma
IgA	Immunoglobulin A
IgG	Immunoglobulin G
Ile	Isoleucine
Leu	Leucine
LNAA	Large neutral amino acids
Lys	Lysine
Met	Methionine
Mil	Million
mRNA	Messenger Ribonucleic acid
NAD	Adenosine dinucleotide
NDF	Neutral detergent fibre
NH_3	Ammonia
NH_4^+	Ammonium
NRC	National Research Council
PBS	Phosphate buffered saline
Phe	Phenylalanine
Pro	Proline
Quin	Quinolinic acid
RNA	Ribonucleic acid
SAPPO	South African Pig Producers Organisation
Ser	Serine

Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
Vit B	Vitamin B

Chapter 1

Introduction

The South African pig industry is a relatively small livestock industry compared to beef, sheep or the poultry industry in South Africa. The commercial pig industry consists of approximately 203 production units with an estimated 110,000 commercia l sows, producing approximately 2.6 million pigs for slaughter per year (SAPPO March, 2014).

Pork producers in South Africa are represented by the South African Pig Producers Organisation (SAPPO). In South Africa, 208 200 tonnes of pork are produced annually, this is low compared to production in Europe and China where 23.06 million and 50 million tonnes of pork per year are produced respectively (FAO, 2012). The *per capita* consumption is also much higher with 40.8 kg per year in European countries and 50 kg in China, compared to only 4.7kg in South Africa (DAFF, 2012). South Africans are traditionally more inclined to consume red meat (beef and mutton), and also poultry because of health concerns and lower cost. There are several communities that do not consume pork due to cultural or religious reasons (SAPPO, 2014). Despite these factors, pork production in South Africa has remained stable and claimed its portion of the protein market.

The breeding industry is characterised by a relatively small number of individual stud breeders that contribute around 27% of local pig genetic resources while two international breeding companies provide between 30% (TOPIGS) and 43% (PIC/Kanhym) of the genetic material respectively (SAPPO, 2014). Pure bred pig breeds availab le in South Africa include the South African Large White, South African Landrace, Hampshire and Duroc. These breeds are used in rotational crossbreeding systems to produce hybrid dam lines with good mothering abilities for commercial production. Pig breeders in South Africa make use of available breeding tools that include animal recording and Estimated Breeding Values (EBVs) and reproductive biotechnologies with up to 80% of all sows in commercial farms making use of artificial insemination (AI).

Pork is mostly produced in intensive production systems that primarily consist of open naturally ventilated housing as well as some environmentally controlled systems. Due to the high cost of building environmentally controlled houses it is mostly the farrowing houses

that are constructed as such. Pigs are produced in all provinces in South Africa with the highest number of pigs slaughtered in Gauteng and Western Cape, closely followed by Kwazulu-Natal. Commercial herds realise reproduction norms of 2.3 farrowings per year, with an average of 13 piglets per farrowing and 25 piglets weaned per sow per year with weaning percentage of up to 92% (SAPPO, 2014). Pigs are slaughtered at 120 days with average carcass weights of 75 kg.

Despite modern housing, feeding technologies and superior genetic resources available, post weaning stress remains a challenge to pig producers worldwide. According to the South African Pork Producers Organization (SAPPO), post weaning morbidity and mortality are significant problems in this country. Post-weaning stress has major economic implications for commercial pig producers resulting in decreased growth rate, poor health and increased mortality. It is therefore important to ensure a stress- free process to reduce post-weaning stress and other development problems.

Post-weaning stress can be defined as the stress or trauma that piglets experience either in a biological, environmental or social level when removed from the dam at weaning (Campbell *et al.*, 2013). In most commercial units piglets are weaned between 23 and 30 days. A considerable amount of stress is experienced by the piglets, not only due to leaving the comfort of the maternal environment, but also the changes in the feed and the adaptation to the new pens and social environment. A number of factors will influence the amount of post-weaning stress such as the piglets' inherent genetic ability and physiological mechanisms to adapt to the new environment. During the weaning process, stressors such as fighting and dominance by larger piglets can be manipulated with good management practises such as grouping of the piglets, to reduce stress and mortality. These practises extend to good vaccination programs, clean water, clean and dry pens and good feeding programmes (Visser, 2014). Despite having good management in place, producers still face challenges with post-weaning stress and unacceptable high post-weaning mortality.

The post-weaning diet of the young piglet can make an important contribution towards the management and reduction of post-weaning stress. At weaning the digestive system of the piglet undergoes structural and immunological changes. The structural changes include mainly villi atrophy and crypt hyperplasia which can lead to poor digestion and absorption of nutrients. This causes the small intestine to be more susceptible to infections (Pluske *et al.*,

1997). The stress that the piglets experience can lead to reduced growth and is also one of the major reasons for post-weaning mortality in a piggery (Pluske *et al.*, 1997; LeDividich & Sevè, 2000).

Antibiotics support the immune system in its defence against bacterial pathogens by decreasing the load of pathogens the immune system has to deal with. In the past, antibiotics were widely included in animal feed. However, the inclusion of antibiotics in feed is currently being limited as a result of pressure from various consumer groups (Stein, 2007; Thacker, 2013).

Amino acids have been shown to play a multifunctional role in the body. The biological contribution of amino acids in the body is a complex process, where 22 amino acids have a role in the formation and function of hormones, enzymes and bioactive proteins, including neurotransmitters and immune modulators (D'Mello, 2003). According to literature there are specific amino acids that have a direct positive influence on the immune system by improving the cells of the intestine in various ways; they are involved in the hormones that stimulate feed intake and are also a fuel source for immunological cells (D'Mello, 2003; Lallès *et al.*, 2009). Amino acids which reportedly have an influence on the immune system include threonine (Thr), tryptophan (Trp) and glutamine (Gln) (Lallès *et al.*, 2009).

It is thus possible that the inclusion of these amino acids in piglet diets at higher concentrations required to sustain optimal growth, may reduce post-weaning stress.

Aim of the study

The aim of this study was to evaluate the effects of threonine, tryptophan and glutamine, alone and in combination, on the performance and immune function of piglets when included in the weaner diet at higher concentrations than currently used in standard commercial rations.

Hypotheses

The following hypotheses were tested during the trial based on the amino acid inclusion only and two different combinations:

Set 1 amino acids:

H_0: Including extra Thr in the weaner diet than the current industry standards will have no effect on piglet performance and the development of the immune system.

H_1 Including extra Thr in the weaner diet than the current industry standards will have a positive effect on piglet performance and the development of the immune system.

H_0: Including extra Trp in the weaner diet than the current industry standards will have no effect on piglet performance and the development of the immune system.

H_1: Including extra Trp in the weaner diet than the current industry standards will have positive effect on piglet performance and the development of the immune system.

H_0: Including extra Gln in the weaner diet than the current industry standards will have no effect on piglet performance and the development on the immune system.

H_1: Including extra Gln in the weaner diet than the current industry standards will have a positive effect on piglet performance and the development of the immune system.

Set 2 combination of two amino acids:

H_0: Including extra Thr and Trp in the weaner diet than the current industry standards will have no effect on piglet performance and the development on the immune system.

H_1: Including extra Thr and Trp in the weaner diet than the current industry standards will have a positive effect on piglet performance and the development of the immune system.

H_0: Including extra Thr and Gln in the weaner diet than the current industry standards will have no effect on piglet performance and the development on the immune system.

H_1: Including extra Thr and Gln in the weaner diet than the current industry standards will have a positive effect on piglet performance and the development of the immune system.

H_0: Including extra Trp and Gln in the weaner diet than the current industry standards will have no effect on piglet performance and the development on the immune system.

H_1: Including extra Trp and Gln in the weaner diet than the current industry standards will have a positive effect on piglet performance and the development of the immune system.

Set 3 combination of three amino acids:

H_0: Including extra Thr and Trp and Gln in the weaner diet than the current industry standards will have no effect on piglet performance and the development on the immune system.

H_1: Including extra Thr and Trp and Gln in the weaner diet than the current industry standards will have a positive effect on piglet performance and the development of the immune system.

Chapter 2

Literature review

2.1 Introduction

The weaning process usually takes place at approximately 28 days of age, when the piglets are removed from the dam. During this time the piglets must rapidly adapt to dramatic changes in their physical and social environments, including switching from milk to solid feed, separation from the sow and littermates and exposure to new surroundings, high stocking densities and unfamiliar piglets (Xiong *et al*., 2015). For the first ten days after weaning the risk for diarrhoeal disease and other problems is at its highest when the digestive and immune systems are not fully developed yet (Visser, 2014). During this time it is important that ideal conditions will be maintained. The house should be at optimal temperature and humidity and be free from draughts and pathogens (Visser, 2014). Literature indicates that poor hygienic housing conditions at weaning contribute to moderate inflammatory responses (Le Floc'h *et al.,* 2007). Furthermore, it has been reported that weaner piglets kept under poor sanitary conditions showed a decrease in feed intake and weight gain (Le Floc'h *et al.*, 2007).

There is a worldwide trend, including in South Africa, to reduce the weaning age of piglets from 28 days to 25 days, and even 21 days. Colson *et al*. (2006) found that weaning at 21 days had more negative consequences on growth rate, stress and endocrine responses than weaning at 28 days of age. Earlier weaning would only be successful under optimal conditions and all factors influencing post-weaning stress should be considered. In this chapter, literature was reviewed with regard to the development of the digestive system at weaning as well as the role of the immune system and feeding regimes at weaning, with specific reference to the role of various amino acids in the development of a healthy piglet.

2.2 The digestive system at weaning

Normal growth and development of animals, including pigs, entail the change in the function of the different organs and the addition of new functions as the body increase in size (Vincek *et al.*, 2012). Growth and development occur at the same time and in synchrony with each other (Lawrence & Fowler, 2002). According to Batt (1980), growth is the physical increase in size whereas development is the change in the body's conformation and shape. The normal growth curve for pigs is shown in Figure 2.1. Although present at birth, the immune system is

not fully developed (Lawrence & Fowler, 2002) and development continoius over time specifically during and after weaning of the piglets. The genetic potential of animals determines the rate and amount of growth that can occur at any stage.

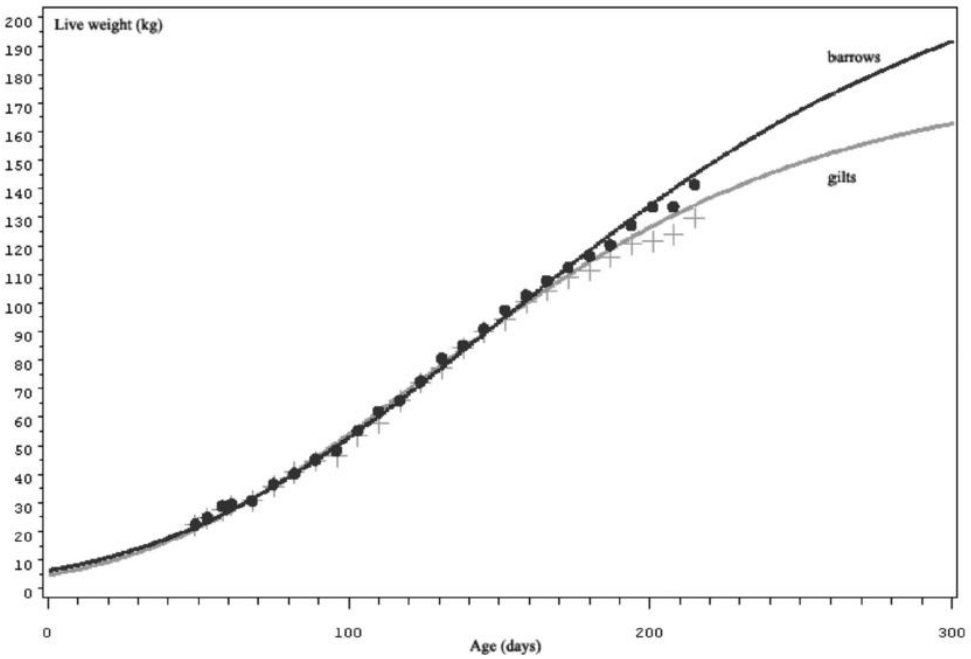

Figure 2.1. Normal growth curve of pigs as illustrated by Vincek *et al.* (2012)

Various biochemical, physiological and histological changes take place during development and growth of the piglet. A number of stressors at weaning further contribute to neuroendocrine and behavioural changes (Prunier *et al.*, 2010) that contribute to a decrease in feed intake and post weaning mortalities. These responses are greater the earlier the weaning occurs (Colson *et al*., 2006). Firstly, the changes in environment when piglets are moved from the farrowing to the weaner house result in fighting and stress. The piglets also need to adapt to a new diet. Risk factors that are associated with high levels of morbidity and mortality during and shortly after the weaning process were indicated by Lallès *et al.* (2004) and are summarised in Table 2.1.

Dietary changes during the time of weaning are inevitable. The piglets no longer receive highly digestible sows' milk and creep feed but a less digestible grain-based weaner feed as their only food source (Lallès *et al*., 2009). Due to the sudden changes of feedstuffs consumed, enzyme secretion is not adapted to the new diet (Prunier *et al.*, 2010) and

digestion and absorption of nutrients are subsequently poor. Lactase activity reduces while maltase and sucrase activity increases gradually during weaning as the digestive tract of piglets adapt to the new feed (Pierzynowski *et al.*, 1993). The low digestive and absorptive capacity of the undeveloped small intestine provides more substrates for pathogen proliferation (Pluske *et al.*, 2002) which creates an optimal environment for microbial overload, especially *Escherichia coli*, *Clostridium perfringens* and rotaviruses. These organisms cause diarrhoea in piglets, including secretory, osmotic and pathogenic diarrhoea (Boudry *et al.*, 2004; Lallès *et al.*, 2004).

Table 2.1 Risk factors associated with the weaning process (adapted from Lallès *et al.*, 2004)

Risk factor	**Potential effect**
Psychological factors	• Separation from dam/mother • Mixing pigs with other litters • Introduced to a new environment (building or even farm)
Dietary factors	• Decrease in the milk content of the feed (liquid diets, highly palatable and digestible) • Only dry feed is being fed (solid, low palatability and digestibility) • Drinking water is supplied separately from unfamiliar drinker systems
Rearing factors	• Large litter sizes causing lower weaning weights • High pig density post weaning • Decrease in housing hygiene • Non optimal environment
Induced intestinal disorders	• Alterations in intestinal morphology and function Morphology – Villus height and crypt depth • Intestinal enzymes – decreased activity • Intestinal absorption, secretion and permeability are disturbed • Associated enteric pathogens • Bacteria (*E.coli*) • Viruses (Rotavirus)

The changes in feeding system cause changes in the small intestine on both a histological and biochemical level. The change from a milk-based diet to a complex plant based diet is also more difficult to absorb. A decrease in feed intake at wea ning has a negative effect on intestinal mucosal function (Dong & Pluske, 2007) due to the effect on the structures of the *villi*. Boudry *et al.* (2004) demonstrated that weaning induces many changes, both acute and long lasting; in the intestine of piglets and that the different segments of the intestine had different responses to weaning. These changes in the small intestine contribute to poor digestion and absorption of nutrients and the post-weaning diarrhoea often seen in the weaner house (Pluske *et al.*, 1997; LeDividich & Sevè, 2000).

Around the time of weaning the villi of the digestive tract undergo changes in their shape and degree of atrophy. The villi change from a long slender shape to a leaf shape and the cells of the villi, the enterocytes, are immature (Vente-Spreeuwenberg *et al.*, 2003). Villous atrophy was observed (Boudry *et al*., 2004) during the first two days after weaning in the duodenum and proximal jejunum but occurred in the ileum only a few days later. The acute changes are more likely due to the multiple stresses imposed on the piglets at the time of weaning. Reducing these stresses by promoting higher feed intake in the early post-weaning period might therefore help to reduce small intestinal structural and functional disorders and diarrhoea post-weaning.

Physical and psychological stressors at weaning increase cortisol release and corticotrophin-releasing factor receptor expression in the intestine of weaned pigs. Paracellular and transcellular permeability and therefore translocation of antigen and bacterial lipopolysaccharides across mucosal barrier are then increased (Moeser *et al*., 2007). The higher permeability of the intestinal barrier allows an influx of potential toxic substances (Vente-Spreeuwenberg *et al.*, 2003) that may contribute to post-weaning mortalities of piglets.

2.3 The immune system

Immunity is defined by Kindt *et al.* (2007) as the protection of the body against pathogens. The immune system can also be described as a multi-cellular system with various different types of cells that need to function together in a complex way to fight off pathogens.

Although the immune system is present at birth and assists the piglet to fight pathogens during early life, it is not yet fully developed (Lawrence & Fowler, 2002). Unlike many other mammalian species, an impermeable placenta separates the foetus from the sow preventing immunoglobulin transfer via the circulatory system (Levast *et al*., 2014). The transition from the sterile uterus to an antigen-rich environment can be life-threatening for neonates. The first level of immunity is obtained when the piglets receive colostrum from the sow. Colostrum is defined as the first milk to be produced by any mammal after giving birth and it is high in maternal antibodies that contribute to early immunity. It is critical that piglets consume colostrum during the first 36 hours after birth because the gut wall in new-born piglets is only briefly permeable to antibodies. New-born piglets deprived of colostrum are extremely sensitive to conventional microflora. Intestinal transport of immunoglobulins into the blood is completed a few days after birth and these maternal immunoglobulins primarily provide local protection (Tlaskalova-Hogenova *et al*., 1994). Immunity obtained from the immunoglobulins in the colostrum is referred to as the passive immunity (McDonald *et al.*, 2002). Because maternal immunoglobulin G (IgG) has a half- life of 14 days, a piglet is passively protected (systemic protection) by maternal colostral antibodies for the first week after birth. IgA from milk, however, provides local protection to the neonates' intestine as long as it suckles from the sow, giving them time to develop their own immune response (Levast *et al*., 2014). Passive immunity only lasts for a short period of time, but is vital for the survival of the piglet during the first few weeks of its life when its own immune system is still under-developed (Roitt *et al.*, 2006; Virdi *et al*., 2013).

During the first few weeks after birth, the active immune system will start to develop and will continue to be active and may undergo changes during the lifetime of the piglet. In Figure 2.2 it is illustrated how the passive immune system weakens as the piglet ages and the active immune system develops over time and taking over from the passive immune system in protecting the piglet. Around the time of weaning the immune systems tend to weaken and this can contribute to post-weaning mortalities experienced in piggeries (Coffey *et al.*, 2000).

The active immune system can be subdivided into the innate and the adaptive immune systems. These two systems must work in conjunction with each other when a pathogen is present. The innate immune system is the first line of defence and less specific than the adaptive immune system. The cells of the innate immune system are always present in the body to eliminate any infection or pathogen when entering the body. When the primary

barriers, the skin or outer layer of organs are intruded, the innate system will start to neutralise the foreign protein immediately. The adaptive immune system follows on the innate immune system and only starts to work a few days after the pathogen has entered the body. These cells recognise the pathogen, adapt so they remember the pathogen and build a memory for the specific pathogen. When the same pathogen enters the body again a faster and more effective line of defence is present, known as the secondary immune response. A diverse amount of cells are generated when there is an adaptive immune reaction, but all the cells generated are for the specific antigen recognised (Coffey *et al*., 2000).

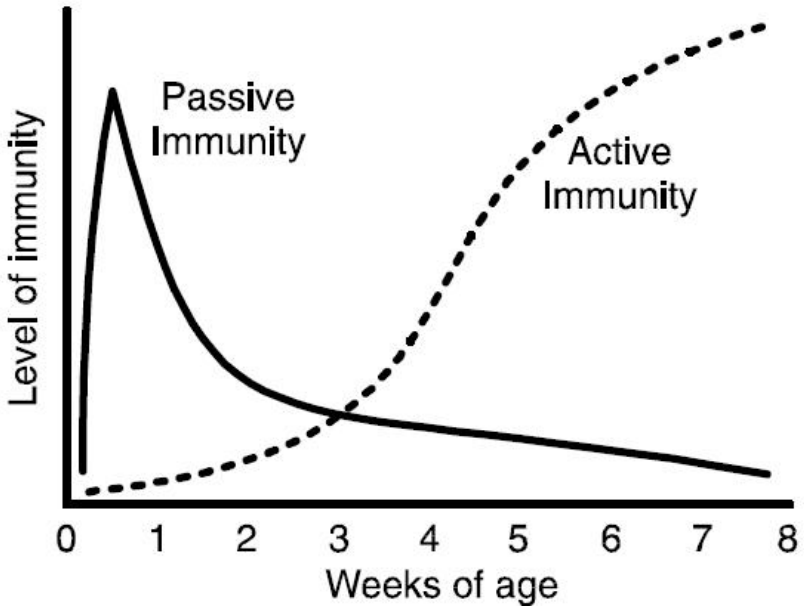

Figure 2.2. Active and passive immunity during the weaning period (Coffey *et al*., 2000)

2.3.1 The development of the immune system

All cells of the immune system originate from stem cells (Kindt *et al.*, 2007). Early on during the *in uterine* development the white blood cells of the immune system originate from the yolk sac. The primary immune cells leave the yolk sac and migrate to the liver and the spleen where these cells colonies. The liver and spleen are therefore regarded as the primary immune organs. Later on during the *in utero* development of the piglet the bone marrow takes over the stem cell differentiation producing immune cells. At the time of birth the bone marrow is the primary organ for development of stem cells for the immune system. The different stages of immunological development pre- and post-birth, as well as the primary organs involved at the specific age can be seen in Figure 2.3.

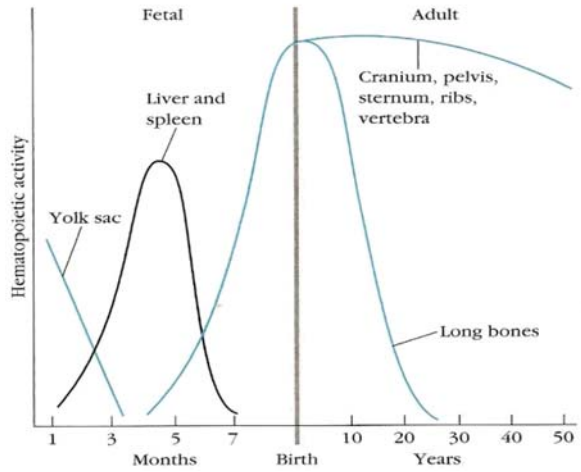

Figure 2.3. The development of the immune system, *in uterine* to adult (Kindt *et al.*, 2007)

The development of the immune system is influenced by growth hormone levels (Hossner, 2005). As the piglet increases in size, its immune system must also grow and develop. Retarded growth can occur when a piglet is exposed to a disease or its immune system is challenged. This retarded growth will affect the competence of the immune system against diseases (Gatnau *et al.*, 1995).

2.3.2 The different cells and organs of the immune system

An antigen is defined as any foreign protein present in the body of an animal (Kindt *et al.*, 2007). These antigens include bacteria, viruses and other pathogens. Antibodies are large proteins present in the bodies of all living animals and they are continuously produced in the body. These antibodies take action against antigens when invading the body.

The organs of the immune system can be subdivided into primary and secondary organs. Immune cell development occurs mainly in the primary organ which are the thymus and the bone marrow. In an immature animal the thymus is large, and functions as the primary immune organ. As the animal matures the thymus shrinks in size; in most adult animals the thymus are not present at all (Lawrence & Fowler, 2002). Figure 2.4 illustrates the shrinking of the thymus over time. The bone marrow takes over as the primary organ for immune cell development in the mature animal (Kindt *et al.*, 2007).

The secondary organs that form part of the immune system are the lymphatic system including all the lymph nodes and the spleen. These secondary organs are responsible for both development and maintenance of the immune system (Kindt *et al.*, 2007).

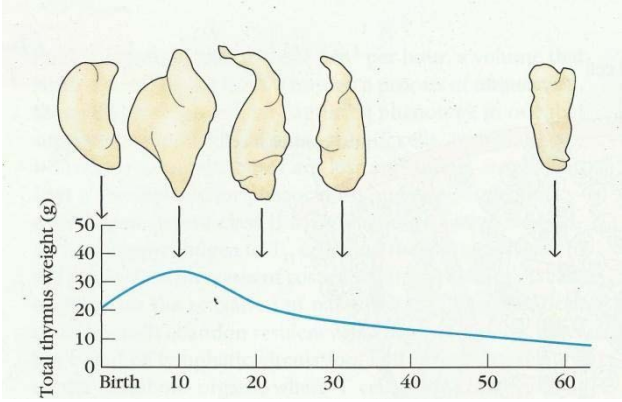

Figure 2.4. Thymus decrease in size as an animal matures (Kindt *et al.*, 2007)

The intestine and the lymphoid tissue in the gut are essential components of the immune system. The intestine and lymphoid tissue are selective permeable. Commensally bacteria and dietary antigens will be tolerated by these organs, where as foreign antigens and pathogens will not be (Ruth & Field, 2013).

The intestine is the largest immune organ in the body. The mucosal epithelium is the primary barrier between the internal milieu and external environment. Externally, the intestine is lined and protected by a layer of unstirred water and a mucus layer, and internally by the tight junctions between enterocytes. These external and internal barriers regulate selective passage of molecules, thereby protecting entry of pathogens and antigens into the system (Farhadi *et al*., 2013). The mucus layer blocks entry of macromolecules such as enzymes and antigens but is permeable to nutrients. It further provides resistance to colonisation of pathogens by adhesion of commensal bacteria in the luminal surface (Montagne *et al*., 2004; Turner, 2009). Mucin, the major macromolecular constituent of mucus, is a glycoprotein secreted by goblet cells. The quantity, viscosity and maturity of mucin covering the epithelial surface are important factors that affect pathogen resistance (Montagne *et al*., 2004). The intestine is the first line of defence for ingested pathogens, therefore it primes naive T- and B- lymphocytes to develop into effector cells and migrate to the intestine as shown in Figure 2.5. (Ruth &

Field, 2013). Cells from both the innate and acquired immune systems are present in the intestine (Wershil & Furuta, 2008).

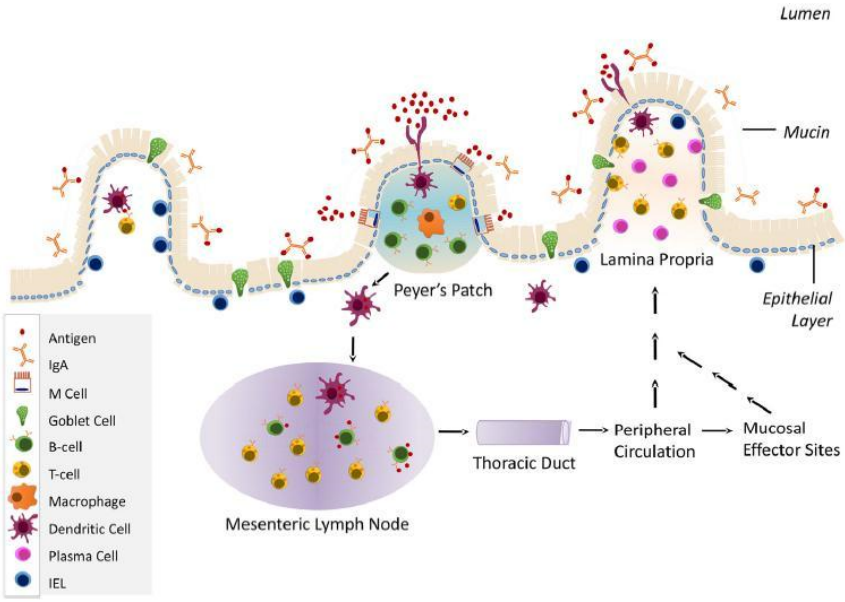

Figure 2.5. Lymphoid tissue in the intestine, the development of lymphocytes and its migration into the intestine (Ruth & Field, 2013)

2.3.4 The response of the immune system to an antigen

The immune system responds in two different ways when a pathogen is recognised (Figure 2.6). The humoral immune response is defined as the immune response that fights extracellular bacteria and macromolecules (Kindt *et al.*, 2007) with its main function the production of antibodies in the plasma, lymph and tissue fluid of the animal. Antibodies, or immunoglobulin, are produced by plasma cells and recognise and neutralise foreign material, such as bacteria and viruses. The antibody recognises a unique part of the target, called an antigen. The antibodies contain paratopes that is specific for one particular epitope on an antigen (Krishnan *et al*., 2008), allowing these two structures to bind together ('lock and key' mechanism). Using this binding mechanism, an antibody can identify foreign material for attack by other parts of the immune system, or can neutralise it directly.

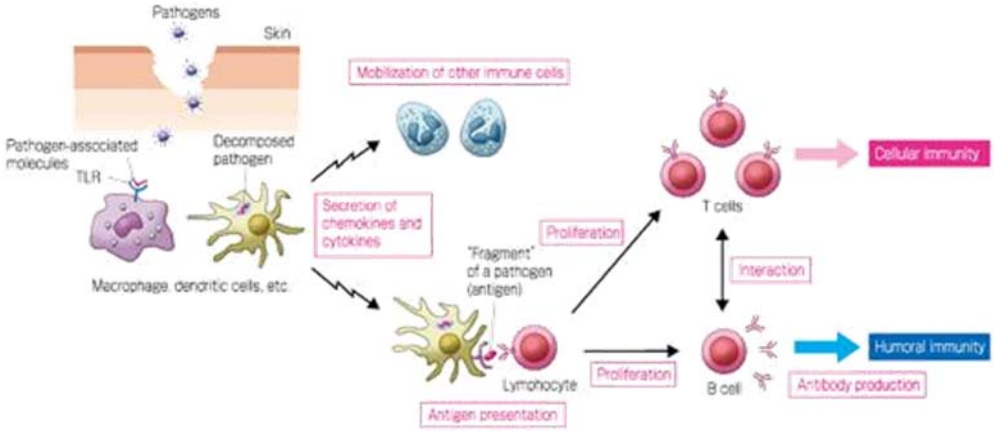

Figure 2.6. The immune system responds in two different ways when a pathogen is recognised (CSLS, 2011.)

Cell mediated response is defined as the response where nonspecific cells of the immune system, such as phagocytes and natural killer cells, as well as antigen specific T- lymphocytes, are present. The cell mediated response protects the body of the animal against intracellular bacteria, viruses and cancer. The two types of lymphocytes, the T- lymphocytes and the B-lymphocytes, are involved in both of these immune responses (Doenhoff, 2000; Kindt *et al.*, 2007).

2.4 Proteins, amino acids and amino acid classification

Body tissues are made up of proteins consisting of 22 different amino acids that can be classified into essential and non-essential amino acids (Pack *et al.*, 2002). Essential amino acids are defined as all the amino acids that must be provided through the diet of the animal as it is not synthesised or stored in adequate amounts in the body. The body does not have the ability to synthesise the carbon skeleton of these amino acids. Non-essential or nutritionally dispensable amino acids are those that can be synthesised in the body of the animal (NRC, 2012). Figure 2.7 illustrates the synthesis of non-essential amino acids from essential amino acids.

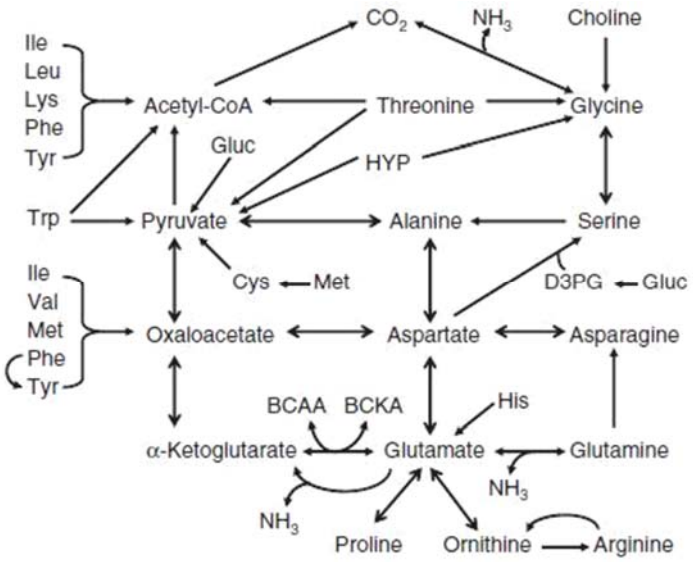

Figure 2.7. The conversion of essential amino acids to non-essential amino acids in pigs (Rezaei *et al.*, 2013)

An additional classification proposed by Boisen *et al.* (2000) refers to semi-essential amino acids that must be included in the diet so that non-essential amino acids can be synthesised. Arginine (Arg), cysteine (Cys) and tyrosine (Tyr) are included in this group of semi-essential amino acids. Arg is synthesised in the urea cycle and Cys and Tyr are synthesised from methionine (Met) and phenylalanine (Phe), respectively.

During certain physiological stages or under specific feeding conditions there may be a higher demand or an insufficient supply of amino acids. It has been reported that the growing pig cannot synthesize sufficient non-essential amino acids to meet their demand at optimal growing and reproductive levels. These amino acids must then be supplied to the animal so that their needs are met. Heger *et al.* (1987); Chung & Baker (1992); Kirchgessner *et al*. (1995) found that the supply of some non-essential amino acids have beneficial effects for growing piglets. These non-essential amino acids that regulate physiological functions via cell signalling pathways include glutamine, glutamate, arginine and proline (Rezaei *et al*., 2013).

The sulphur containing amino acids and aromatic amino acids must be supplied to the animal through its diet. These two groups are important for the synthesis of non-essential amino acids. Amino acids can also be further classified as functional amino acids, which can be

defined as amino acids that are involved and regulate key metabolic functions in the body including health, survival, growth, development and reproduction (Jankowski *et al.*, 2014). Included in this group of functional amino acids are essential amino acids (Arg, Cys, Gly, Leu, Met, Pro, Trp, and Tyr), conditional essential amino acids (Gln) as well as the non-essential amino acid, Asp.

The microorganisms in the rumen of ruminants do have the capability to synthesise the essential amino acids, and ruminants are therefore less dependent on the diet as a source of essential amino acids (D'Mello, 2003). However, the feed of all monogastric animals, including the piglet, must contain all essential amino acids in sufficient quantities. The diet must also contain sufficient nitrogen for the synthesis of non-essential amino acids. The most effective nitrogen source in monogastric nutrition is a mixture of non-essential amino acids (Boisen *et al.*, 2003; NRC, 2012). Piglets, unlike ruminants, are not capable of utilising free nitrogen to make amino acids. It is therefore important to supply the piglets with the correct ratio and levels of amino acids for them to grow at their genetic potential. The amino acids are classified in Table 2.2 according to the body's ability to synthesise it.

Table 2.2. The essential and non-essential amino acids (Pack *et al*., 2002)

Essential amino acids	**Non-essential amino acids**
Lysine (Lys)	Glutamate (Glu)
Histidine (His)	Glutamine (Gln)
Leucine (Leu)	Glycine (Gly)
Isoleucine (Ile)	Serine (Ser)
Valine (Val)	Alanine (Ala)
Methionine (Met)	Aspartate (Asp)
Threonine (Thr)	Asparagine (Asn)
Tryptophan (Trp)	
Phenylalanine (Phe)	

Various interactions exist between the different amino acids. Some amino acids may not be stored or synthesised in the body from the primary building blocks, but may be synthesised from other amino acids. An example of this is arginine (Arg) that is synthesised from glutamine (Gln). The synthesised Arg is still inadequate for the requirements of the animal,

because most of the Arg is catabolised in the urea cycle in the liver. Therefore, additional Arg must be taken in through the diet (Fuller, 1994). The branch-chain amino acids, valine (Val), isoleucine (Ile) and leucine (Leu) are also interdependent, and the supply of one affects the other. The relationship between methionine (Met) and cystine (Cys) is a very important and complicated relationship. Both these amino acids contain sulphur. Met must be supplied to all animals in their feed. Both Cys and Met are incorporated into the DNA of animals. In pigs and some other species Cys is classified as a conditional essential amino acid. The metabolic pathway of converting Met to Cys requires adenosine triphosphate (ATP) and vitamin B as activator and coenzyme, respectively. The Met and Cys relationship is a one way relationship, meaning that Met can be converted to Cys, but not the other way around (Pack *et al*., 2002).

2.4.1 The metabolis m and absorption of proteins

Food proteins must be transformed into tissue proteins that involve complex biochemical and physiological processes (Figure 2.8). The transformation process includes the digestion, absorption and metabolism of amino acids. Enterocytes, microorganisms in the lumen of the small intestine, digestive organs and multiple signalling pathways are involved (Rezaei *et al*., 2013).

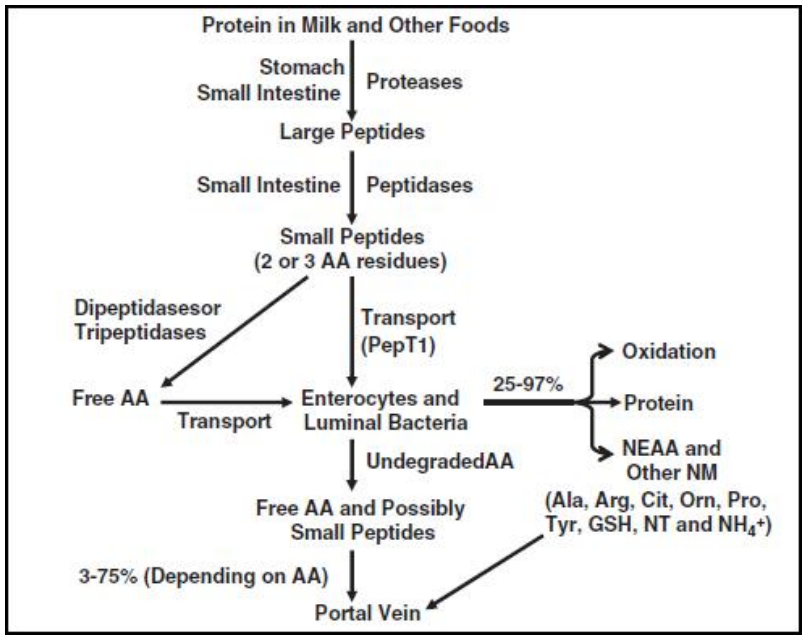

Fig 2.8. The catabolism of proteins into amino acids (Rezaei *et al*., 2013)

The proteins that are ingested via the feed are exposed to HCl and peps in in the stomach of the animal. Once the proteins and polypeptides enter the small intestine the pancreatic proteases break these components down to even smaller components. Peptides are broken down in the intestinal tract by intraluminal enzymes to free amino acids and peptides. Only glutamate, glutamine and aspartate are extensively degraded in the small intestine (Li *et al*., 2010). Enterocytes absorb the free amino acids immediately so it can be used in the body. The smaller peptides are readily absorbed into the enterocytes. Amino-peptidases in the brush borders of the intestinal wall must break down the larger peptides before they can be adsorbed (Krehbiel & Matthews, 2003).

Amino acid metabolism occurs in different places in the body. The major organs for amino acid metabolism are the gut, muscle, liver and the brain. Amino acids cannot be stored in the body and must be catabolised when not used as amino acids. The catabolic products of amino acids differ between animal species and amino acids. Ammonia is formed in fish, urea in mammals and uric acid in birds and reptiles as end products of amino acid catabolism (D'Mello, 2003; Brosnan & Brosnan, 2006).

2.4.2. Protein quality

The availability and the digestibility of the amino acid content of any feedstuff are considered the main components when evaluating protein quality in monogastric animal nutrition (Nahashon *et al*., 2011). Different protein sources have different digestibility and amino acid availability for pigs. This is important to consider when formulating pig diets. There is a difference in quality between animal and plant protein sources. Proteins from animal origin are usually of better quality than plant protein sources, but over- heating any protein source during processing decreases the quality of the protein severely. The most readily available plant protein to pigs is heat-treated soybeans. Soybeans must be processed before they can be fed to pigs due to the anti- nutritional factors present in the raw soybeans. Protease inhibitors present in the soyabeans will not only affect the digestion of the soyabeans but also the digestion of the other feed ingredients (McDonald *et al.*, 2002).

A diet must be formulated according to the digestible amino acid values for a specific raw material as not all the amino acids are digestible and available to the animal. The total crude

protein content of a diet can be decreased and the utilisation of the protein in the diet can be increased by supplying the correct amino acid balance (Mosenthin & Rademacher, 2003). Proteins can also be evaluated according to their biological value. Proteins with a high biological value are referred to as a protein that has a high content of the essential amino acids. If any of the essential amino acids are missing the biological value of the protein is nil. A protein with an almost perfect amino acid balance will have a high biological value (D'Mello, 2003).

The ideal protein can be defined as the protein that contains the perfect amino acid balance but also contains enough additional nitrogen in order for the non-essential amino acids to be synthesised (Cole & Van Lunen, 1994). The first- limiting amino acid in the diet of animals is the amino acid that would be the first deficient amino acid which would limit the rate of protein synthesis. It is generally accepted that lysine (Lys) is the first- limiting amino acid in most piglet feeds (NRC, 2012). This is especially true when the feed is maize- rather than wheat-based, because Lys is present in limited amounts in maize. The most common protein source in piglet feeds is soyabean which contains a low percentage of the amino acid methionine. Amino acids available and the required ratios of the different amino acids in a diet are often expressed as a proportion of Lys, because Lys is generally the first limiting amino acid in typical pig rations. Lys is also the most studied amino acid in all animal diets (Lewis, 2001; D'Mello, 2003).

2.4.3 Role of amino acids in the immune system

Amino acids have a multifunctional role in the body that goes far beyond protein synthesis for the formation of body tissues. Other functions include activities such as cell signalling and the regulation of gene expression (mRNA translocation) and protein phosphorylation (Wu, 2009; Wu, 2010). All 22 amino acids are also involved in the formation and function of hormones (melatonin and thyroxin), enzymes and bioactive proteins, including neurotransmitters (γ-amino-butyrate, dopamine and serotonin) and immune modulators (D'Mello, 2003; Heger, 2003; Wu, 2009). They also play a role in ammonia removal and metabolic regulation.

Almost all of the amino acids have a direct or indirect effect on the immune system (Li *et al*., 2007). Animals reared in an environment in which they are exposed to environmental antigens, or those affected by disease or internal parasites, have reduced appetite and growth, suggesting modifications of nitrogen and amino acid metabolism. Amino acids are redirected away from production towards cells involved in the immune response. Immune system activation results in the synthesis of specific proteins that play crucial roles in the defence of the animal against pathogens and the modulation of immune response. These changes in amino acid metabolism induced by the activated immune system alter the required amino acid profile. The question is whether these higher requirements for specific individual amino acids could be met with by increasing its concentration in the diet to allow the simultaneous preservation of skeletal muscle protein, performance of the growing animal and efficient immune response (Le Floc'h *et al*., 2004). Cytokines, especially interleukin-1, inferno γ, tumour necrosis factor and interleukin-6, are involved in protein metabolism regulation and might be mediators that cause changes in amino acid metabolism during immune activation and inflammation (Marinkovic *et al*., 1989; Cooney *et al*., 1994). Acute-phase proteins such as fibrinogen, C-reactive protein and haptoglobin are synthesised and secreted by the liver through induction of interleukin-1 and interleukin-6 (Gruys *et al*., 1998). Reeds *et al*. (1994) established that these proteins are rich in aromatic amino acids (phenylalanine, tyrosine and tryptophan) and much higher than that found in muscle protein (growth).

The sulphur-containing amino acids, methionine and cysteine, may also have an influence on the inflammatory response in the body (Takahashi *et al.,* 1997; Grimble, 2006). Jankowski *et al*. (2014) found that an increase in the Met concentrations of broiler starter and grower diets to 1.2% and 0.9%, respectively, have stimulated the immune system without improving the broilers' growth and feed efficiency. Cysteine is an important constituent of acute-phase protein but is also used for glutathione synthesis involved in the intracellular detoxification of free radicals (Grimble & Grimble, 2006). Furthermore, cysteine is an important building block of mucin, which is an important barrier of pathogens and their toxins in the lumen of the gut. Disease- induced stimulation in mucus secretion leads to an extra requirement for cysteine.

It has been shown that arginine can reduce inflammation of the gut induced by enterotoxins, reduce bacterial translocation, improve acute-phase protein synthesis, stimulate bactericidal

actions of macrophages and enhance macrophage phagocytic activity (Lallès *et al*., 2009). Arginine also enhances the proliferation of T- lymphocytes stimulated by mitogens (Le Floc'h *et al*., 2004).

In the current study, the effect of higher intakes of tryptophan, glutamine and threonine, on the immune system of weaner piglets were investigated and for this reason, these three amino acids are reviewed in more detail in the following sections.

2.4.4 Threonine

Threonine (Thr) is one of the essential amino acids for pigs. Depending on the main ingredients the diet is based on, it is usually the first to the fourth limiting amino acid in the diet. Threonine has important roles in the maintenance requirements of the pig as well as in supporting gut health and the immune system (Defa *et al.*, 1998). The majority of the dietary Thr is absorbed in the ileum. The chemical structure of Thr is illustrated in Figure 2.9.

Threonine is the most abundant essential amino acid in immunoglobulin protein (Bowland, 1966; Liu & Putman, 1979; Low *et al*., 1979). Defa *et al*. (1998) found that Thr-deficient growing pigs had lower plasma concentrations of immunoglobulins after immune response activation. Cuaron (1984) found that increased percentages of Thr in the diet of sows increased IgG levels in the blood when fed a sorghum based diet. Li *et al.* (1999) and Wang *et al.* (2006) had similar findings than Cuaron (1984) when bovine serum albumin and ovalbumin were injected into growing piglets. When Li *et al*. (1999) increased the concentration of Thr in the diet of the pigs there was a higher production of humeral antibodies and IgG. Where Lys is the first limiting amino acid for nitrogen retention; it appears that Thr may be the first limiting amino acid for immunoglobulin production. Wang *et al.* (2006) concluded that for 10-25 kg pigs, 5.9 g of true ileal digestible Thr per day might be sufficient for maximum growth rate and feed conversion, but this should be increased to 6.6 g per day to optimise immunity. Abbasi *et al*. (2014) have found that the inclusion of higher Thr concentrations (110% of Ross specifications) in the diets of broilers had no effect on Newcastle antibody titres but the antibody titres against sheep red blood cells were significantly improved during both primary and secondary responses. Similarly, Ren *et al*. (2014) found that standard recommended concentrations of Thr were adequate for growth and

feed conversion in weaned piglets. However, higher concentrations of Thr promoted the function of immune organs (spleen and thymus) as well as the secretion of IgA in the gut mucosa after challenge with *E.coli*. In the same experiment, lymphocyte response to challenge under mitogen was also higher for weaned piglets that received the high concentrations of Thr.

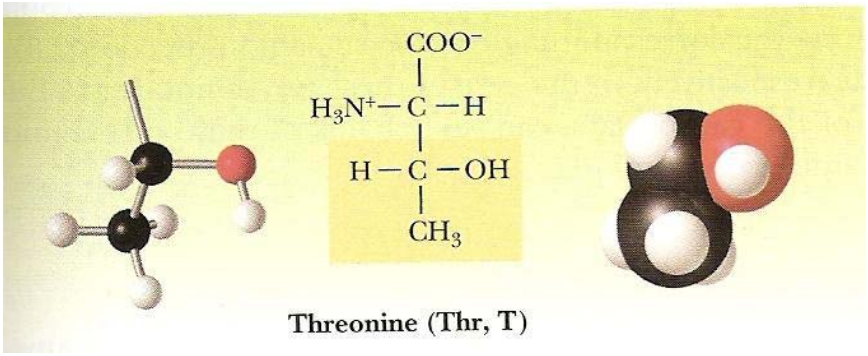

Figure 2.9. Structural illustration of threonine (Campbell & Farrell, 2003)

Furthermore, Thr requirement is strongly associated with intestinal metabolism. Stoll (1998) and Bertolo (1998) indicated that the fraction of Thr absorbed in the ileum is not entirely delivered to the portal vein for utilisation by the various body functions. Instead, the digestive tract itself uses a significant portion of the absorbed Thr for gut growth, structure and function (Hoskins, 1984; Bertolo, 1998; Law *et al*., 2000; Ball, 2002; Jansman *et al*., 2002). A more recent study done by Law *et al*. (2007) concluded that plasma Thr was lower when a Thr deficient diet was fed compared to an adequate diet.

Mucus, a digestive secretion, contains high concentrations of Thr. An adequate intake of dietary Thr is crucial for mucus production and maintenance of gut function in piglets and therefore important in the integrity of nonspecific defences of the gut wall (Carlstedt *et al*., 1993). This Thr is not re-absorbable, resulting in endogenous losses being rich in Thr (Le Bellego *et al*., 2002).

Ren *et al*. (2014) found that villi height of the duodenum and jejunum decreased in weaned piglets after *E.coli* challenge and that additional Thr in the diet allowed a widening of duodenum *villi* and a decrease in jejunal *villus* height to crypt depth ratio.

Threonine deficiency may also be associated with increased intestinal para-cellular permeability and reduced ileal villi height in young piglets (Hamard *et al*., 2007) and broilers (Abbasi *et al.*, 2014).

However, Wang *et al*. (2007) found that an excess of Thr had a negative effect on intestinal protein synthesis, perhaps as a result of its competitive inhibition on the absorption of other indispensable amino acids. Keene (2001) and Edmonds & Baker (1987b) found that supplemental feeding of Thr had a negative effect on body weight gain and feed intake in chickens. Excess of dietary Thr also caused reductions in the growth rate and feed intake in post weaned rats (Muramatsu *et al*., 1971; Castagné *et al*., 1995; Sarwar, *et al*. 1995; Castagné *et al*., 1996) and pigs (Edmonds *et al.,* 1987). This negative effect of a high Thr in the diet on growth rate may be caused by an imbalance of amino acid intake and not through additional specific toxic effects (Castagné *et al*., 1996).

2.4.5 Tryptophan

In a maize-based diet, tryptophan (Trp) appears to be the second limiting amino acid after Lys (Burgoon *et al*., 1992). Figure 2.10 shows a chemical representation of Trp. According to Guzik (2002) when the body tissue of pigs are analysed, Trp is present at the lowest concentration when compared to the other amino acids.

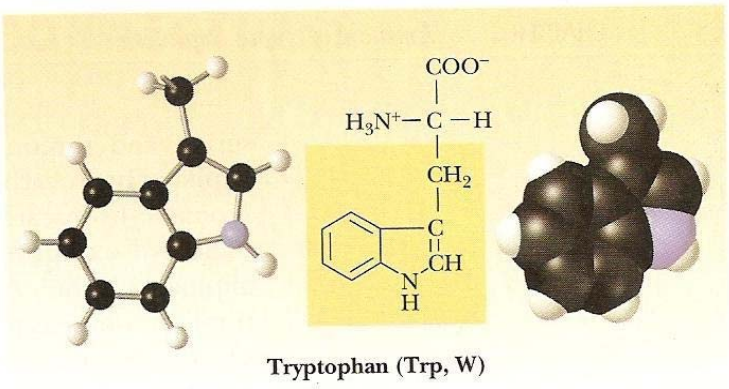

Figure 2.10. Structural illustration of tryptophan (Campbell & Farrell, 2003)

Trp has various other functions beyond muscle protein synthesis. Trp appears to improve the efficiency of growth, development and reproduction in pigs. Trp can be transported albumin bound (90%) or free (10%). Only the free Trp can cross the blood-brain barrier. In the brain

the free Trp can be either involved in protein synthesis, serotonin synthesis, tryptamine synthesis in the neurons or kynurenine and kynuramin synthesis (Chen & Guillemin, 2009).

Trp is a precursor for the neurotransmitter in the brain known as serotonin (Fig 2.11). The Trp that enters the brain through the blood brain barrier is converted to serotonin (Madras *et al.*, 1974). This conversion in the brain mainly occurs in the serotonergic nerves, enterochromaffinic cells, thrombocytes and mast cells (Fernstrom & Fernstrom, 1995. Serotonin is widely distributed in the hypothalamus (Saavedra *et al*., 1974). Serotonin's role is to regulate feed intake and body temperature, but it also has an influence on stress resistance, mood control and animal behaviour, including impulse, aggressive and sexual behaviour (Leathwood, 1987; Sainio *et al*., 1996; Sevè, 1999). It was indicated by Henry *et al*. (1992) that diets with low Trp percentages resulted in a depressed feed intake. An increase of the Trp specification for a weaner diet (additional 5 g/kg feed) enhanced the recovery of piglets from 'social stress' after mixing of litters at weaning by reducing cortisol and noradrenaline concentrations in the blood. Tryptophan may therefore also spare arginine and glutamine catabolism in enterocytes of post-weaning pigs since cortisol stimulates the degradation of arginine and glutamine (Flynn & Wu, 1997; Lallès *et al*., 2009). It is possible that Trp and Arg improve gut integrity, digestion and tissue anabolism during stressful periods such as weaning by inhibition of the adreno-cortical and sympatho-adrenal axis activity (Lallès *et al*., 2009).

In the pineal body serotonin is metabolised to a neuro- hormone, melatonin. Melatonin is involved in the control of day- night-rhythms. Melatonin is also involved as an intracellular scavenger of peroxide radicals and hydroxyl radicals (Reiter *et al*., 1994).

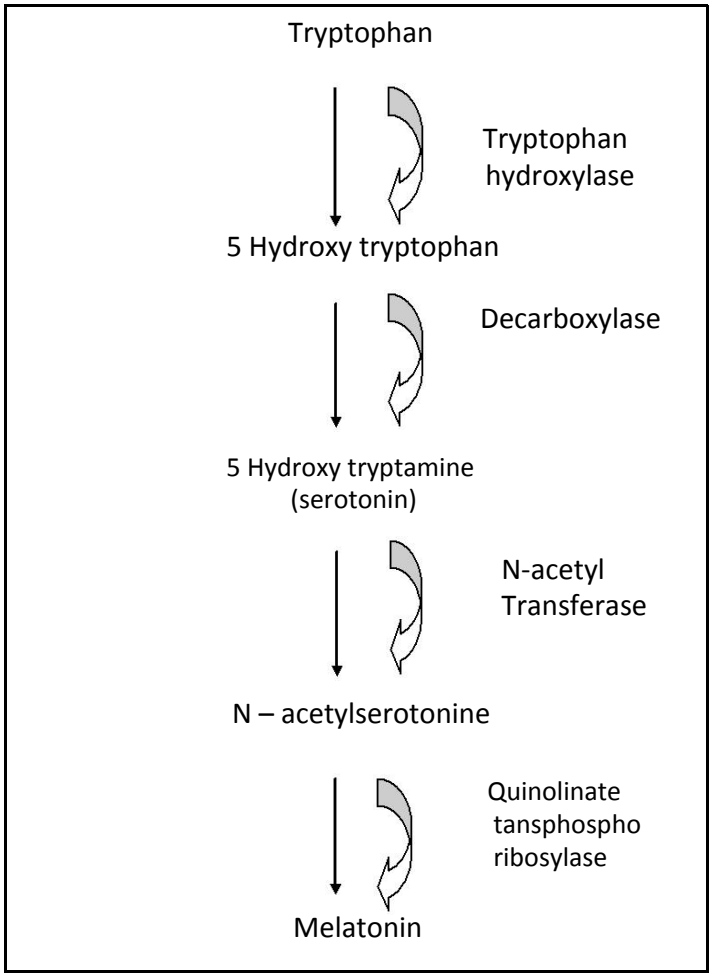

Figure 2.11. Ilustration of the metabolic pathway of tryptophan to serotonin and melatonin synthesis.

Reeds *et al*. (1994) suggested a high Trp requirement for acute-phase protein synthesis with stimulation of the immune system. Trp can also be linked to the immune system via the kynurenine pathway (Moffett & Namboodiri, 2003) (Fig 2.12). The kynurenine pathway occurs in the astrocytes, microglial, macrophages and dendritic cells in the brain of the pig. With the help of enzymes and cofactors Trp is oxidised to nicotinamide adinosine dinucleotide (NAD) in the kynurenine pathway (Chen & Guillemin, 2009). NAD is defined as a coenzyme that is involved in several oxidation-reduction reactions in the body (Blood & Studdert, 1999). Pfefferkorn (1984) found that Trp was degraded at a faster rate when T cells released interferon gamma (IFN-γ) during an immune response. Some of the kynurenines, quinolinic acid (QUIN) and 3-hydroxyanthranilic acid (3HAA) are selective immune molecules that can be activated during an immune reaction (Chen & Guillemin, 2009). During an inflammatory or immune reaction increased QUIN levels was also observed by Moffett *et al*.

(1997). Trevisi *et al*. (2009) supplemented a standard weaner diet with 100 mg/kg L-tryptophan and found an improvement in daily gain and feed intake in piglets susceptible to intestinal adhesion of *E.coli* K88 during the first 4 days after the challenge. Le'Floch *et al*. (2004) showed that plasma Trp concentrations in pigs declined and kynurenine concentration increased after induction of lung inflammation and suggested that in pigs, Trp requirement may be increased during immune system stimulation.

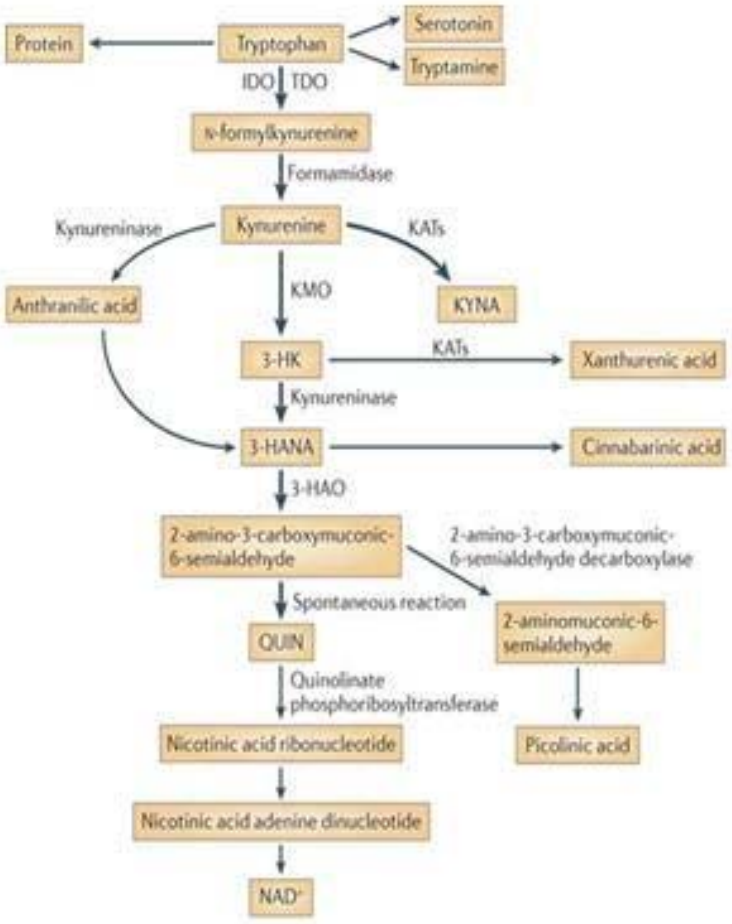

Figure 2.12. The oxidation of tryptophan to NAD via the Kynurenine pathway (Schwarcz *et al*., 2012)

2.4.6 Glutamine (Gln)

In general glutamine (Gln) is abundant in proteins and physiological fluids. It is also a key regulator in gene expression and cell signalling pathways (Rhoads & Wu, 2009). By supplementing adequate amounts of dietary Gln, maximum growth, development and production performance in pigs can be supported (Wu *et al.*, 2011).

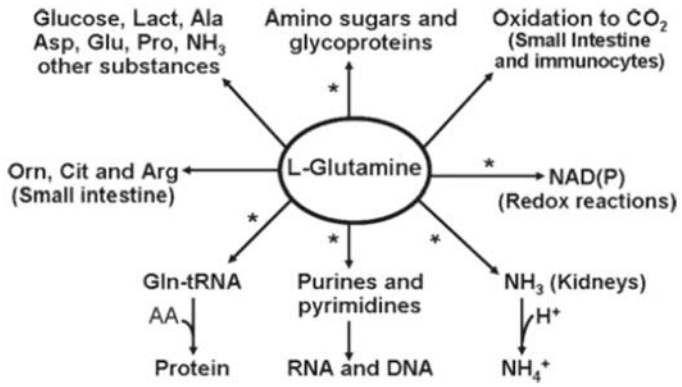

Fig 2.13. Multiple functions of Gln in the body (Wu *et al*., 2011)

Glutamine, usually classified as a non-essential amino acid, also has a considerable impact on the development and maintenance of the immune system of weaner piglets and can therefore be considered a conditionally essential amino acid (Castell, 2002) under inflammatory and disease conditions (Cabrera *et al*., 2013). According to D'Mello (2003) both intestinal mucosal cells (enterocytes) as well as intra-epithelial lymphocytes require Gln as an energy source. Gln is also needed for the synthesis of other non-essential amino acids and nucleotide bases. According to Schrock & Goldstein (1981), Gln is a precursor for increased renal ammoniagenesis during chronic metabolic acidosis. Gln is also essential during purine and pyrimidine biosynthesis where its amide N is used. The multiple functions of Gln are summarised in Figure 2.13 and its chemical structure can be seen in Figure 2.14.

At weaning and when intestinal stress is experienced Gln may become limiting. The limited Gln seen at weaning is due to rapid turnover and replacement of mucosal cells in the intestine (Wu *et al*., 1996). By feeding increased levels of Gln at weaning the immune system and the gut health of the piglets might be improved, while the amount of diarrhoea seen at weaning might be reduced (Wu *et al*., 1996). It might also increase the amount of nutrients absorbed, which will improve overall feed efficiency and growth rate (Johnson *et al*., 2006).

Gln is not just an energy source for intestinal cells but is also important in the function, maintenance and structure of the cells in the intestine (Ruth and Field, 2013). Gln is the only amino acid that can be absorbed from arterial blood into the small intestine of pigs. The small intestine utilise 30% of arterial Gln and 67% of dietary Gln (Wu *et al*., 1994). Wu *et al.* (1996) has shown that supplementing 1% Gln in post weaner diets prevented villi atrophy in

the small intestine of infected, early-weaned piglets. Likewise, Cabrera *et al*. (2013) supplemented creep feeds with 1% Gln and found significantly longer villi and more proliferating cells in the jejunum of these piglets.

B- and T- lymphocytes, neutrophils and macrophages are dependent on Gln (Newsholme, 2001). Yoo *et al*. (1997) reported that proliferation of blood lymphocytes was significantly higher in *E. coli*–infected piglets when fed a diet containing 40 g glutamine/kg compared to no supplementation. Domeneghini *et al*. (2004) found that 5 g Gln/kg feed increased ileal mucosa densities of macrophages and intra-epithelial lymphocytes. Furthermore, 40 g/kg Gln supplementation stimulated lymphocyte proliferation to mitogens and cytokine response of T-lymphocytes present in mesenteric lymph node cells of piglets (Johnson *et al*., 2006). It is, however, not clear whether the immuno-stimulation by glutamine is direct or indirect via the extra energy provided to the immune system (Bannink *et al*., 2006).

Glutamine's classification has been changed from the traditional classification as non-essential to conditional essential (Castell, 2002) because it was found that Gln has a positive effect on the animal during stressful times, such as weaning. Gln can therefore be regarded as an important amino acid at weaning.

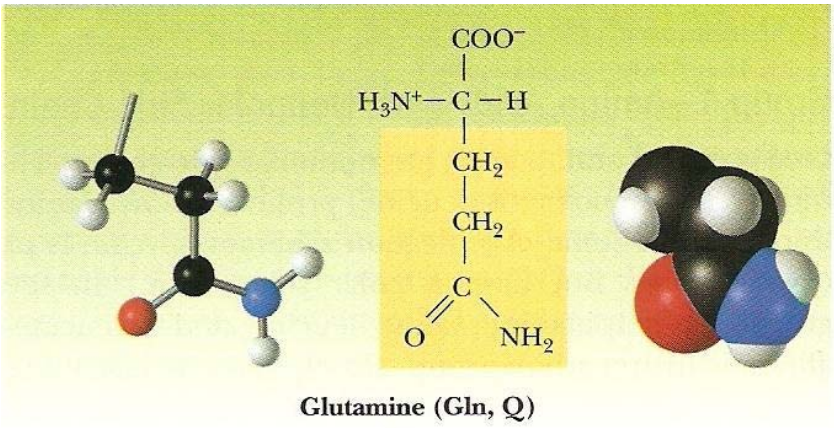

Figure 2.14. Structural illustration of glutamine (Campbell & Farrell, 2003)

2.5 Amino acid require ment of the weane r pig

Most producers aim to optimise the post-weaning environment of the young piglet in such a way as to decrease the amount of stress and therefore limit post-weaning mortalities. The composition of the feed used during this period plays a major role in post-weaning performance and the development of the immune system. Amino acid availability is a major contributor in the development of the immune system (Sandberg *et al*., 2006).

The change in feed composition at weaning has an effect on the changes of digestive enzymes that occur at weaning. The major changes that occur are the decrease in lactose and the increase in starch as the main source of carbohydrates to the piglets (Pluske *et al.,* 1997) that may result in diarrhoea. The weaner's feed must be of such that it limits the occurrence of diarrhoea, and cause as little as possible digestive upsets.

Different environmental conditions will have an influence on amino acid requirement. A pig will also have different amino acid requirements during different life stages (Pack *et al.,* 2002). A pregnant sow, for instance, has a different requirement of amino acids than a dry sow. Weaner piglets' amino acid requirements also differ from that of grower and finisher pigs. When a study is done to determine the requirement of a specific amino acid, all the other amino acids must be supplied in sufficient amounts, so they do not cause limitations during the study.

Amino acid requirements can be divided into priority for usage. First priority for animals is maintenance; this is the requirements an animal has to maintain normal body function (Hossner, 2005). Once the maintenance requirements are met, the requirements for growth, lactation and reproduction will receive priority. Amino acids must be supplied in the correct ratio to make them all available and usable to the animal. All amino acids that are present in excess have no value to the piglet, except that the amino acid is broken down and the carbon is used as an energy source. This catabolism of amino acids is an energy-expensive and an undesirable process (McDonald *et al*., 2002).

Conclusion

Many factors contribute to post weaning stress and mortalities in a piggery. According to the literature findings, when feeding the piglet the most optimum feed with a perfect amino acid balance, the amount of stress that the piglets experience could decrease. There is an interaction between specific amino acids and the immune system of piglets. Inclusion of the optimum dietary concentrations of Thr, Trp and Gln may improve the immune competence of piglets after exposure to antigens which may decrease post weaning mortalities. These concentrations are, however, not yet established and research in this regard is necessary.

Chapter 3

Materials and Methods

3.1 Introduction

In this study the influence of three amino acids on growth and immunity were evaluated in piglets shortly after weaning. Different levels and combinations of threonine, tryptophan and glutamine were included in the diets of piglets from around weaning for a total period of four weeks. Ethical approval was obtained from the Animal Use and Care Committee of the University of Pretoria (EC045-11) as well as from the University of KwaZulu-Natal (088-11-Animal) before the onset of the trial. The trial was conducted at the Ukulinga Research Farm of the University of KwaZulu-Natal, Pietermaritzburg, South Africa.

3.2 Materials

Animals

A total of 48 crossbred weaned piglets (Landrace x Large White) were purchased from Swineline piggery in the Kwamhlanga district in Gauteng, South Africa. The piglets were collected from Swineline early in the morning of day zero of the trial. They were transported by road in an enclosed truck, to prevent a draught. The temperature was not controlled, but was monitored during the trip to ensure that the piglets were comfortable and subjected to as little stress as possible. Hay bedding was provided to the piglets to keep them warm and comfortable during the eight hour journey to Pietermaritzburg.

All piglets were weaned at 28 days of age, but at the time of purchase 50% of experimental animals were a week older than the others. At commencement of the trial, the group of piglets comprised of 12 males of 39 days of age, 12 females of 39 days of age and 24 females of 32 days of age. The piglets were clearly marked according to age. At placement, the piglets were randomly allocated to eight treatments, with three piglets of each age group per treatment (6 replicates in total per treatment). Male piglets were randomly allocated so that each treatment had either one or two male piglets.

Experimental pig unit

The experimental unit at the Ukulinga Research Farm of the University of KwaZulu-Natal, Pietermaritzburg, South Africa, is an open-sided pig facility of 8 m x 25 m, fitted with curtains and slatted floors. One week before onset of the trial the unit was washed with a high pressure washer and disinfected with commercial chemicals. The pens, curtains and self-feeders were also cleaned and disinfected. The individual pens (60 cm x 100 cm) of the piglets had plastic slatted floors, suspended from the floor to allow urinary, faecal, water and feed wastage to fall through. The sides of the units were made from expanded metal. Three individual pens were grouped together to form a unit as illustrated in Figure 3.1. This was done to ensure that social interaction was possible between the piglets. A chain suspended from the roof was provided for each pen for social enhancement. The feed was provided using plastic self- feeders placed in front of each individual pen. There was a nipple drinker in the back of each pen (Figure 3.1).

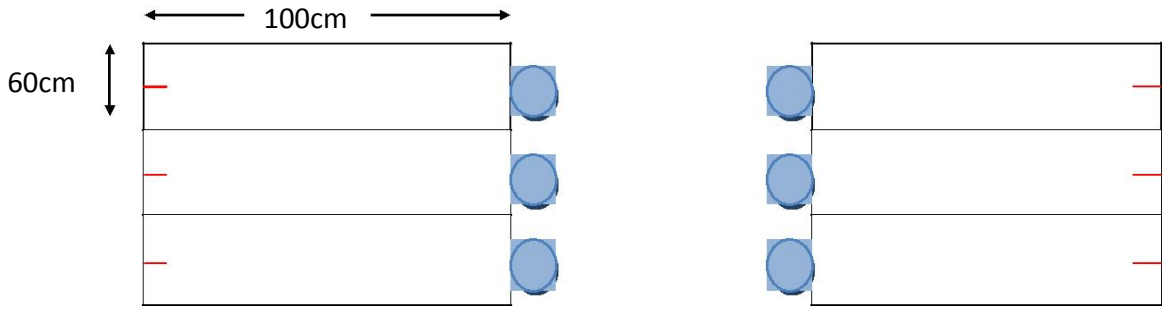

Figure 3.1. A schematic representation of the pen lay–out. Blue dots represent the feeders and red lines the position of the nipple drinkers

Temperature regulation

The optimal temperature for weaner pigs is approximately 21^{o}C to minimise weaning stress (Visser, 2014). The pig unit was preheated using a gas heater blower during the morning of day zero of the trial to have a temperature of approximately 21°C on arrival of the piglets. This heater was used throughout the trial to regulate the temperature of the house, in combination with the curtains. In the front, middle and back of the house the temperature was monitored on an hourly basis by means of digital thermometers. At Day 23 to Day 26 of the trial, an extreme cold spell was experienced with outside temperatures dropping below 0°C and snowfall occurred. Temperature fluctuations inside the pig house were inevitable during

this time. The piglets in the different sections (front, middle and back) of the pig unit were, however, all subjected to the same fluctuations and temperature did not vary between the sections in the unit (Figure 3.2).

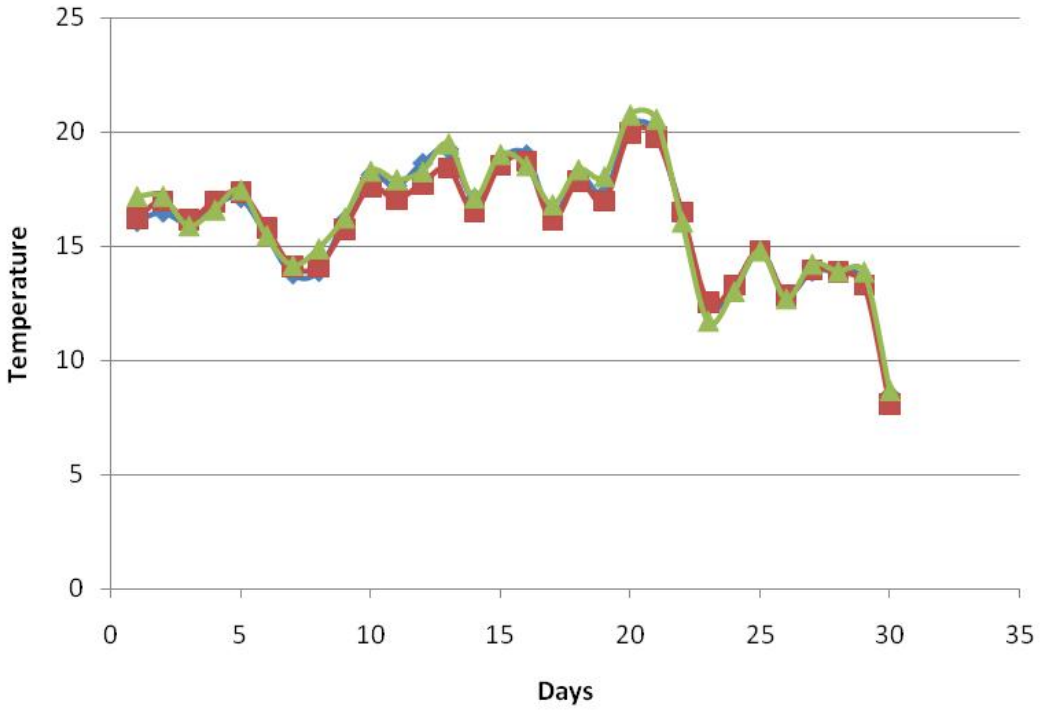

Figure 3.2. The average temperature in the different sections of the house during the 28-day trial period. The different areas in the house are represented by: Front Middle Back

3.3 Treatments

The basal diet used in the trial was a standard commercial pig weaner diet. Treatment 1, which served as the negative control group in the study, received the basal diet (Table 3.2) without any extra amino acids added. Threonine (Thr), tryptophan (Trp), glutamine (Gln) and combinations of these amino acids were added to the basal diet for the different treatments. A total of eight treatments were included in the study, each replicated six times. Treatments 1-8 are shown in Table 3.1.

Table 3.1. The different experimental treatments included in this study

Treatments	**Description**
Treatment 1 (None)	Control diet (Commercial weaner diet)
Treatment 2 (+ Thr)	Control + Threonine
Treatment 3 (+ Trp)	Control + Tryptophan
Treatment 4 (+ Gln)	Control + Glutamine
Treatment 5 (+ Thr + Trp)	Control + Threonine + Tryptophan
Treatment 6 (+ Thr + Gln)	Control + Threonine + Glutamine
Treatment 7 (+ Trp + Gln)	Control + Tryptophan + Glutamine
Treatment 8 (+ Thr + Trp + Gln)	Control + Threonine + Tryptophan + Glutamine

The feed was analysed for crude protein (CP) and moisture content in the Animal and Poultry Science laboratory at the University of KwaZulu-Natal. Gross energy (GE) and neutral detergent fibre (NDF) were also analysed at the same laboratory for calculation of digestible energy (DE) using the following equation:

DE = (3.77- (0.019*(NDF*10)) + (0.758*GE), where NDF is in % and GE and DE are in MJ/kg. (R.M. Gous. 2011. Personal communication, University of Kwazulu-Natal)

The nutrient composition of the basal diet is shown in Table 3.2. A commercial formulated ration for weaner piglets were used.

Table 3.2 Analyses and calculated (digestible energy) chemical composition of the basal commercially purchased diet

Nutrient	**Value**
Crude protein (g/kg)	200
Moisture (g/kg)	124
Gross energy (MJ/kg)	13.9
Digestible energy (MJ/kg)	12.7
Neutral detergent fibre (NDF) (g/kg)	8.5

The amino acid profile of the basal feed were analysed at the Department of Animal and Poultry Science, University of KwaZulu-Natal, using a Beckman 6300 analyser (Beckman Coulter, California, United States of America) according to standard procedures described by the manufacturer. The sample was prepared by means of the standard protein hydrolysis procedure. An internal standard was added to the sample as a correction factor (Moore & Stein, 1948). The amino acid composition of the basal feed is shown in Table 3.3.

The tryptophan content of the basal feed was analysed by the Agricultural Research Council, Irene. An enzymatic hydrolysis method was used for analysis. An HPLC was used for separation and detection of the amount of Trp using a fluorescence detector. The p re-set specifications was LOQ = 0.006 g/100g and LOD = 0.002 g/100g.

Table 3.3 Amino acid composition of the basal feed (DM basis) (g/100g)

Amino Acid	**Content (g/100g)**	**Amino Acid**	**Content (g/100g)**
Cysteine	0.299	Valine	0.787
Methionine	0.733	Isoleucine	1.306
Aspartic acid	1.757	Leucine	1.334
Threonine	0.758	Tyrosine	0.749
Serine	0.855	Phenylalanine	1.397
Glutamine	0.311	Lysine	1.624
Proline	1.649	Histidine	0.479
Glycine	0.889	Arginine	1.118
Alanine	0.848	Tryptophan	1.296

Only the basal (control) feed was analysed in the laboratory. Amino acids are usualy expressed in relation to Lys. This is done because Lys is the first limiting amino acid in pig diets. Table 3.4 shows the relationship between Lys and the other amino acids.

Table 3.4 Amino acid profile, expressed as essential amino acids relative to lysine, for the basal feed (based on total amino acid values) and compared to recommended ideal amino acid profiles (based on standardised ileal digestible values) for young pigs (12-25 kg)

AA:Lys	**NRC (2012)**	**INRA[1]**	**Basal feed of this experiment**
Lys:Lys	100	100	100
Thr:Lys	**59**	**65**	**47**
Met:Lys	29	30	45
(Met + Cys):Lys	55	60	64
Trp:Lys	**16**	**22**	**80**
Val:Lys	63	70	48
Ile:Lys	51	52-60	80
Leu:Lys	100	101	82
Phe:Lys	58	54	86
(Phe + Tyr):Lys	93	-	132
Tyr:Lys	-	40	46
His:Lys	34	31	29

[1]Gloaguen *et al*. (2013)

The inclusion levels of the three test amino acids were calculated by means of the following equation:

Amino acid content of the basal feed + 1.5 g amino acid/100g feed added to the different feeds.

The calculated amino acid values are shown in Table 3.5.

Table 3.5 Calculated amino acid concentrations of the three amino acids in the different treatments

Treatments	Threonine (g/100g)	Tryptophan (g/100g)	Glutamine (g/100g)
Treatment 1 (None)	0.758	1.296	0.311
Treatment 2 (+ Thr)	2.258	1.296	0.311
Treatment 3 (+ Trp)	0.758	2.796	0.311
Treatment 4 (+ Gln)	0.758	1.296	1.811
Treatment 5 (+ Thr + Trp)	2.258	2.796	0.311
Treatment 6 (+ Thr + Gln)	2.258	1.296	1.811
Treatment 7 (+ Trp + Gln)	0.758	2.796	1.811
Treatment 8 (+ Thr + Trp + Gln)	2.258	2.796	1.811

Preparation of the different treatment diets

One week before the onset of the trial the feed was mixed in a clean and dry one ton feed mixer. The mill was cleaned out before and in between mixing different treatments to prevent any cross contamination. The feed was stored in a clean, dry storage room on wooden pallets (Figure 3.3, marked A). A total of 38 kg of the basal diet was weighed off on a digital scale for each treatment (Figure 3.3, marked B). An extra 2 kg of the basal feed was weighed for every treatment. This was placed in a separate container (Figure 3.3, marked C). An amount of 600 g of the relevant amino acid(s) was added to the 2 kg of basal feed and mixed well for 2 minutes in a plastic bag, by shaking the bag in the different directions (Figure 3.3, marked D). The 38 kg basal feed together with the feed-amino acid mix were added to the 1 ton mixer and mixed for 10 min (Figure 3.3, marked E). The feed was bagged, clearly marked and stored in the store room (Figure 3.3, marked F).

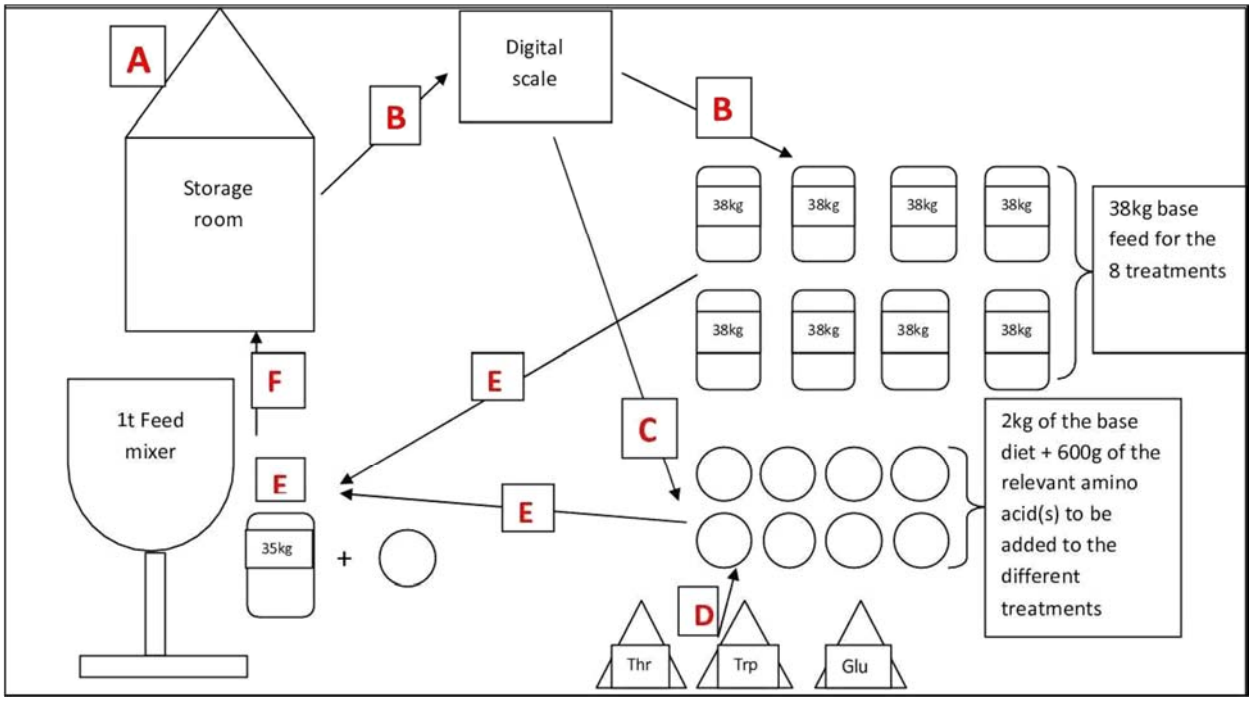

Figure 3.3. A schematic representation of the feed mixing procedure followed

3.4 Procedures and data collection

Placement of the piglets

On arrival at Ukulinga Research Farm the piglets were offloaded and placed in a holding camp to adjust to the new environment. The piglets were individually weighed on a digital walk- in scale. The piglets were identified with an ear tag that was numbered in numerical order. The piglets were placed into their different age and sex groups from where they were randomly allocated to individual pens where the self- feeders were already filled with a specified weighed amount of the treatment feed. The feed and water were provided *ad libitum* to the pigs during the trial. Once all the piglets were weighed and settled in their individual pens they were vaccinated with 1 mL of a 10 % ovine red blood cell suspension by intramuscular injection. The ovine red blood cell vaccination was given to the piglet to challenge their immune system with a foreign protein they have not been exposed to previously.

Preparation of 10 % ovine red blood cell suspension

During the morning of day 0 the ovine blood was collected from the jugular vein of a sheep. The blood was collected in a blood tube containing heparin. The sheep blood was centrifuged at *1200 g* for 5 min to separate the erythrocytes and plasma. The ovine erythrocytes were washed three times with phosphate buffered saline (PBS: pH7.4) centrifuging the blood in between each wash. Finally, a 10 % ovine red blood cell suspension was prepared using the washed erythrocytes and PBS (Tinker & Gous, 1986).

Body weight

The piglets were weighed on days 0 and 28 using an electronic walk-in scale. Each piglet walked from its pen onto the scale and its weight was recorded. The piglet was then placed back in his/her individual pen.

Feed intake

Feed intake (FI) data were collected by first weighing the empty self- feeder and then adding the feed before it was weighed again. Self- feeders were weighed at regular intervals on day 7, 14, 21 and 28. The orts were collected in trays that were placed underneath the self- feeder and were also weighed on days 7, 14, 21 and 28.

The feed intake was then calculated on a weekly basis as follows:

FI/pig/week = Feed weight (day 1) – feed left (day 7) – orts/week

Feed intake was determined weekly for accuracy reasons. There was a concern that a high degree of wastage would occur from orts pans at the bottom if the feed was kept in the feeders for 28 days. By measuring the feed intake weekly the feeders could also be cleaned weekly and filled with fresh feed.

The accumulative feed intake for the 28 day trial was calculated by adding the weekly feed intakes together.

Feed conversion efficiency (FCE)

Feed conversion efficiency (FCE) was calculated for the trial for each pen.

The FCE was calculated as follow:

FCE/pig/trial = Feed intake accumulative / weight gain of the pig during trial

Determination of antibody values against ovine erythrocytes

Blood from the jugular vein of each piglet was collected on days 7, 14, 21 and 28. The piglets were restrained by placing them on their backs on a wooden plank; the head was slightly pulled back and held down. The blood was collected in heparin- free tubes with a new needle for every piglet. The blood tube was numbered according to trial day and piglet number.

Serum samples were then immediately collected by centrifugation of the blood at 1200 *g* for 5 minutes to separate the serum and erythrocytes. The serum was collected with an eppendorf pipette in a sterile plastic tube containing 30 µL Phosphate Buffered Saline (PBS). The tube number corresponded with the piglet number and day of collection. The tubes were placed in numerical order in polystyrene tube holders and stored in a fridge at 4^{o}C until further analysis. Antibody values against ovine erythrocytes were determined in microtitre plates using passive haemagglutination (Tinker & Gous, 1986; Nicholson *et al*., 1993) at the Vet Diagnostics Laboratory in Pietermaritzburg.

After completion of the trial, the stored serum samples from each piglet were incubated in a warm water bath for 30 minutes at 55.5°C to inactivate complement. Serum samples were then washed three times with PBS by centrifuging at 1200 *g* for 5 min. This was to remove any erythrocytes that might have been included in the serum sample. Fresh ovine blood was collected in heparin containing blood tubes. The blood was collected from the same sheep that were used for inoculation of the piglets. The sheep blood was centrifuged at

1200 g for 5 min to separate the erythrocytes and plasma. The ovine erythrocytes were washed three times with phosphate buffered saline (PBS: pH7.4) and re-suspended at 2 % haematocrit in PBS.

Each well of a 96 V-bottomed microtitre plate was filled with 125 µl PBS. In well 2 of the microtitre plate, 125 µl serum was added. After mixing, 125 µl out of well 2 was added to well 3. This process was continued through to well 12 so that a decreasing pig serum solution was obtained. 25 µl of the 2 % sheep erythrocyte solution was then added to all 96 wells. The microtitre plate was incubated with its lid closed at room temperature on a shaker for 2 hours. All the serum samples were analysed in duplicate. Figure 3.4 is a schematic representation of the described procedure for determining antibody values in the serum.

The antibody titer value was taken as the highest dilution where haemagglutination was visible. The 1:1 dilution was given a value of 1, while the 1:2 dilution a value of 2, 1:4 a value of 3. The wells without serum served as negative controls.

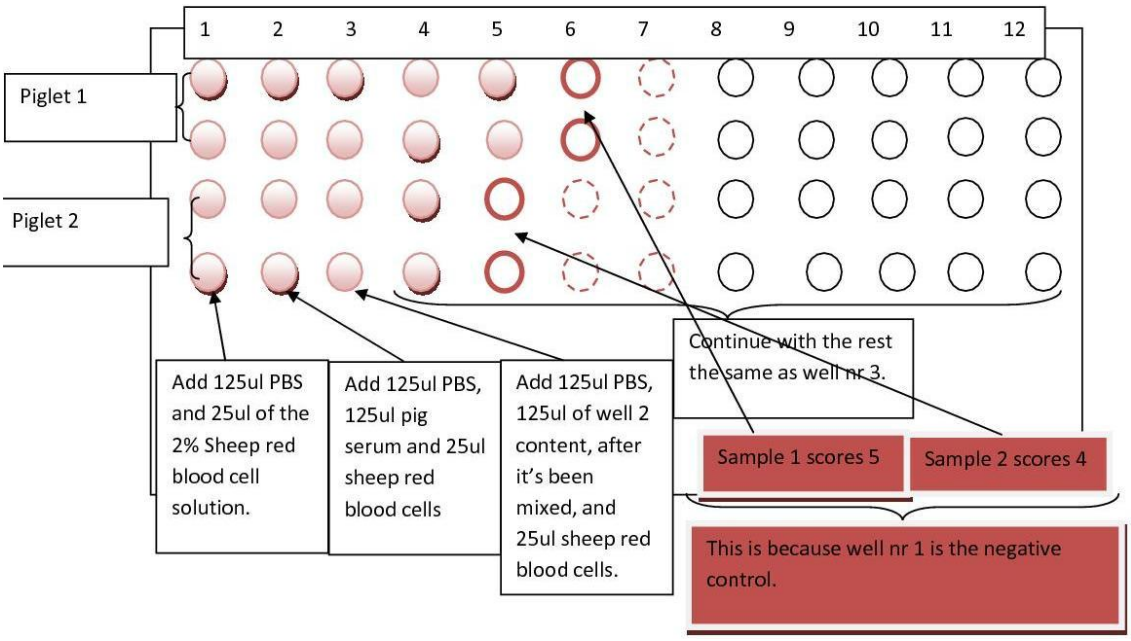

Figure 3.4 A schematic representation of determining antibody titres against ovine erythrocytes

3.5 Statistical analysis

Regression analysis

A simple linear regression analysis was done on the total weight gain data for each individual piglet using Genstat, 12th Edition (Procedure Library Release PL20.1). Weight on day 0 was added as a covariate. The results obtained from the individual regression analysis were used

in an ANOVA analysis where the means and standard errors were calculated for body weight gain.

ANOVA analysis

A standard analysis of variance (ANOVA) was performed on the total feed intake and FCE data. Treatment means, standard errors and least significant differences of the means were determined using Genstat software, 12th Edition (Procedure Library Release PL20.1). Weight on day 0, sex and age were tested as potential covariates in both data sets.

Chi square analysis

A Chi square analysis was used for weekly titre values of the different treatments. The maximum score for each week was determined for each treatment. The total weekly score for each treatment was determined by summing the individual titre scores of the pigs on each treatment. A Chi square analysis was conducted using the total measured score and the total possible score for each treatment.

Chapter 4

Results

4.1 Introduction

Results are presented for body weight gains, feed intake and FCE of piglets reared on weaner diets containing different concentrations and combinations of Thr, Trp and Gln during a 28 day growth trail. Antibody titres were analysed to evaluate the immune response to a challenge with ovine erythrocytes.

4.2 Body weight gains, feed intake and FCE

Mean body weight gain, feed intake and FCE of piglets on each treatment, obtained by regression of total weight gain, feed intake and FCE over time, are provided in Table 4.1.

Piglets that received the diets that contained additional Thr (Treatment 2) had significantly lower average daily body weight gains ($P<0.05$) than the piglets from all the other treatments. Feed intake did not differ between any of the treatments although Treatment 2 (+ Thr) showed a numerically lower feed intake. The addition of Thr to the basal diet also resulted in a significantly lower feed efficiency in the piglets ($P<0.05$), compared to all the other treatments. The lower body weight gain and feed efficiency were only noted for piglets that received additional Thr (Treatment 2) and not when the higher Thr intake was combined with higher concentrations of Trp and/or Gln (Table 4.1).

Table 4.1 Body weight gain, feed intake and feed conversion efficiency (FCE) of weaner piglets that received higher concentrations of Thr, Trp and Gln or combinations thereof over a 28-day trial period

Treatment	Weight gain, g/d	Feed intake, g/d	FCE, g gain/kg feed
Treatment 1 (None)	463[a]	846	549[a]
Treatment 2 (+ Thr)	407[b]	810	503[b]
Treatment 3 (+ Trp)	485[a]	887	554[a]
Treatment 4 (+ Gln)	496[a]	922	539[a]
Treatment 5 (+ Thr + Trp)	484[a]	946	514[a]
Treatment 6 (+ Thr + Gln)	426[a]	840	508[a]
Treatment 7 (+ Trp + Gln)	477[a]	865	549[a]
Treatment 8 (+ Thr + Trp + Gln)	493[a]	899	554[a]
Mean effect of Thr	452	874	538
Mean effect of Trp	485	899	543
Mean effect of Gln	473	882	538
SEM: main effects	12.5	23	10.1
SEM: two-way int.	17.9	33	14.5
SEM: three-way int.	25.3	46.6	20.5

[a,b] Means within columns with differed superscripts differed significantly (P< 0.05) SEM: Standard error of the mean

4.2 Antibody titres

Blood was collected on day 0 and then weekly thereafter during the 28 day trial period. Antibody titres of piglets that received the ovine red blood cells did not differ significantly from each other between treatments ($P<0.05$) (Table 4.2).

Table 4.2 Mean weekly antibody titres of weaner piglets receiving supplementary Thr, Trp and Gln or combinations thereof over a 28-day trial period

Treatment	**Antibody titre**				
	Day 0 (collection 1)	**Day 7 (collection 2)**	**Day 14 (collection 3)**	**Day 21 (collection 4)**	**Day 28 (collection 5)**
Treatment 1 (None)	0	14	16	8	4
Treatment 2 (+ Thr)	0	20	12	13	7
Treatment 3 (+ Trp)	0	15	14	7	6
Treatment 4 (+ Gln)	0	14	10	7	7
Treatment 5 (+ Thr + Trp)	0	10	6	10	12
Treatment 6 (+ Thr + Gln)	0	10	14	8	7
Treatment 7 (+ Trp + Gln)	0	13	11	3	6
Treatment 8 (+ Thr + Trp +Gln)	0	11	11	4	8
Chi Square	0	3.91	4.62	7.80	3.90
P-Value	0	0.79	0.71	0.35	0.79

As shown in Figure 4.1 to Figure 4.4, the titre values of all treatments followed the same trend. All the values started at 0, had a steep increase during the first 7 days followed by a

steady decrease. The addition of Thr (Treatment 2) resulted in the highest titre values on days 7 and 21 of the trial, where the combination of additional Thr and Trp (Treatment 5) showed the highest titre values at the end of the trial, 28 days after the challenge with ovine erythrocytes.

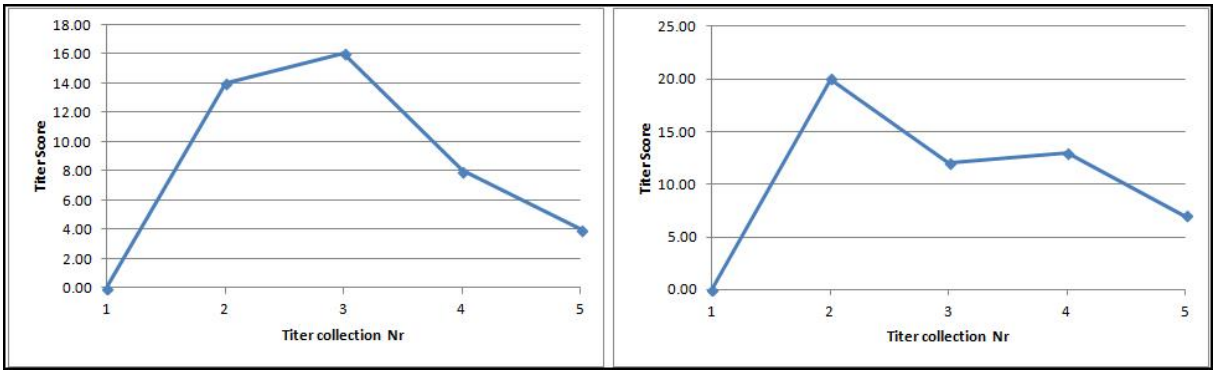

Figure 4.1 Graphic representation of the titre values of Treatment 1 and Treatment 2, respectively, over the 28 day trial period

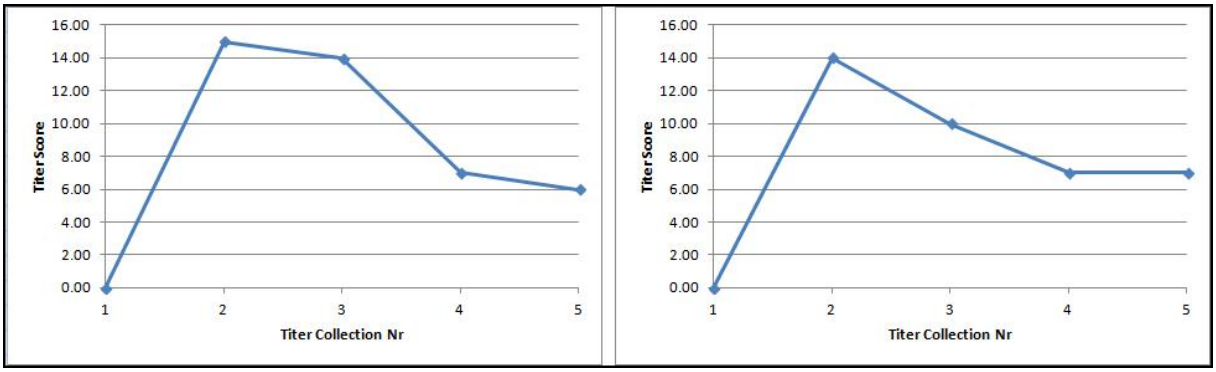

Figure 4.2 Graphic representation of the titre values of Treatment 3 and Treatment 4 , respectively, over the 28 day trial period

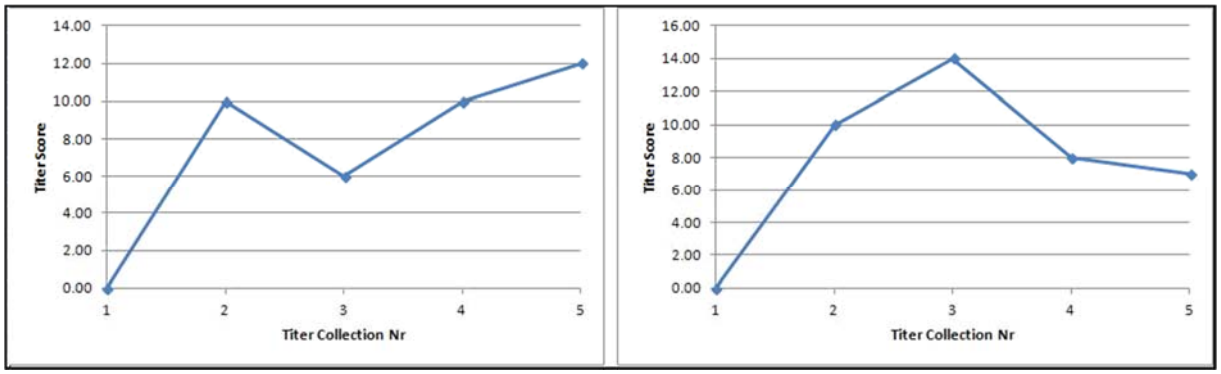

Figure 4.3 Graphic representation of the titre values of Treatment 5 and Treatment 6, respectively, over the 28 day trial period

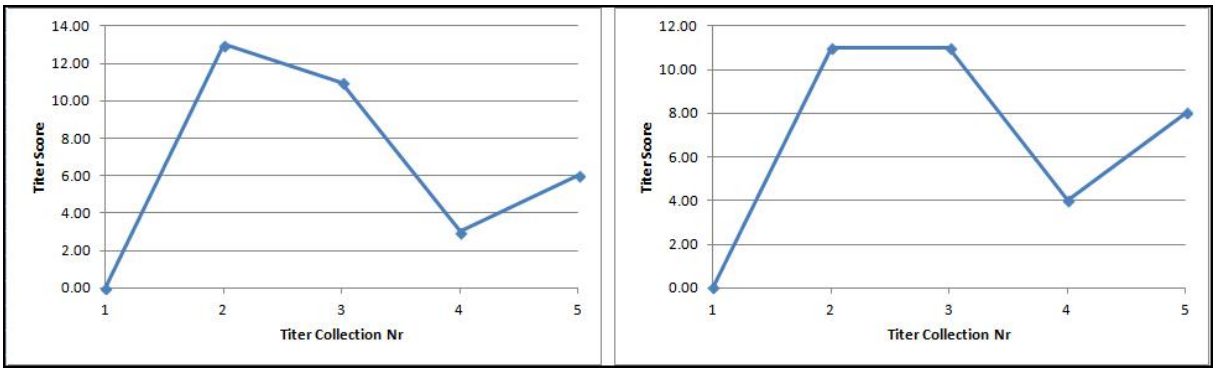

Figure 4.4 Graphic representation of the titre values of Treatment 7 and Treatment 8 respectively over the 28 day trial period

In Figure 4.5 the treatments are presented in one graph and the titre values follow the same trend except for Treatment 1(+Thr) that had higher values at day 2.

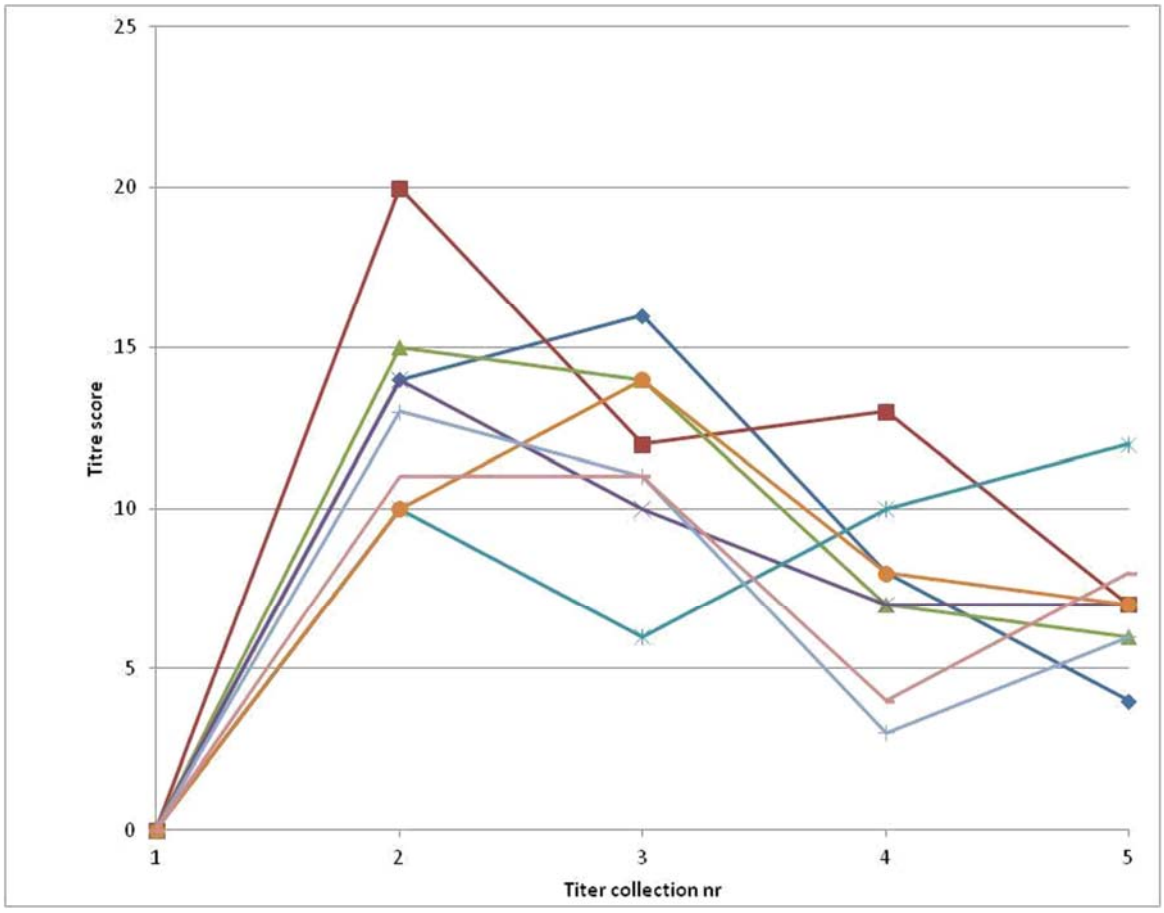

Figure 4.5 A graphic representation of the antibody titre values of weaner piglets that received higher concentrations of Thr, Trp and Gln or co mb inations thereof over a 28 -day trial period

Chapter 5

Discussion

Post weaning stress in piglets is a major concern for pig producers worldwide. Commercial farmers loose profits due to decreased growth, additional veterinary costs and increased mortality post weaning. Several studies have highlighted the factors associated with post weaning stress (Campbell *et al*., 2013). It is evident that diet changes are a major contribution to post weaning stress.

Amino acids have been shown to play a multi- functional role in the body. The biological contribution of amino acids in the body is a complex process, where 22 amino acids have a role in the formation and function of hormones, enzymes and bioactive proteins, including neurotransmitters and immune modulators (D'Mello, 2003). Specific amino acids have a direct influence on the immune system by improving the cells of the intestine in various ways; they are involved in the hormones that stimulate feed intake and are also a fuel source for immunological cells (D'Mello, 2003; Lallès *et al.*, 2009). Amino acids reported to have an influence on the immune system include threonine (Thr), tryptophan (Trp) and glutamine (Gln) (Lallès *et al.*, 2009).

In this study the aim was to evaluate the effects of threonine, tryptophan and glutamine on the immune function when included in the weaner diet of piglets at higher concentrations than currently used in standard commercial weaner rations.

5.1 Post weaning growth, feed intake and efficiency

The growth of the piglets measured as average daily gain varied between 407 g/day and 496 g/day among the treatments. The addition of Thr to the basal feed resulted in the lowest body weight gain of 407g/day, which was significantly lower than that of Control 1 (463 g/day), the diet with no additional amino acids. A similar result was obtained by Keene (2001) where supplemental feeding of Thr in chicken diets also had a negative effect on body weight gain and feed intake. Excesses of dietary Thr above 1% of the diet content induced reductions in the growth rate and feed intake in post weaned rats (Muramatsu *et al*., 1971; Castagné *et al*., 1995; Sarwar *et al*., 1995). Body weight of rats from conception to death at 90 days of age

was reduced (Castagné *et al*., 1995) when the rats were fed a diet containing four times normal Thr levels. The chick seems to be more sensitive than the pig towards an excess of 4% of dietary Thr as the growth rate in the chick were reduced by 29% (Edmonds & Baker, 1987b) compared to the 5% in pigs (Edmonds *et al*., 1987). In early work on the effects of amino acid supplementation, Edmonds and Baker (1987a) and Castagné *et al*. (1996) were of opinion that elevated levels of amino acids in pig feed caused a decrease in feed intake and growth rate due to an imbalance of the amino acids and not antagonistic or toxic effects. However, Wang *et al*. (2007) found that an excess of Thr had a negative effect on intestinal protein synthesis, perhaps as a result of its competitive inhibition on the absorption of other indispensable amino acids. This effect might also have contributed to a decrease in growth rate in the pigs that received higher levels of Thr in their diet.

Studies done by Le Bellego *et al.* (2002) and Wang *et al.* (2007) suggested that Lys: Thr ratio had an influence on growth rate in pigs. They found that when the Lys: Thr ratio was low, body weight gain was low as well. Thr must be 65% of the Lys content in a piglet's diet for optimal performance according to a review by Ajinomoto (2002). Furthermore, De Blas *et al.* (2000) found that the digestibility coefficient of Thr is lower than that of Lys, because the speed of hydrolysis and absorption is slower for Thr.

Combining high levels of more than one amino acid in the diet resulted in more positive effects. Russel *et al*. (1983) found that supplementing Thr and Trp simultaneously had a positive effect on body weight gain in piglets as well as in the feed:gain ratio. In this study, combining higher dietary concentrations of Thr with Trp and Gln (Treatment 8) resulted in higher body weight gain (493 g/day).

Low feed intake is directly correlated to low body weight gain. Feed intake varied between the treatments from 810 g/d to 946 g/d. In this study the lowest feed intake was observed when Thr (Treatment 2) was added to the diet ($P>0.05$). This correlated with the results for the lowest body weight gain. In rats as well as in rhesus monkeys, a high protein diet also caused a decrease in feed intake (Harper & Peters, 1989; Hannah *et al*., 1990; Jean *et al*., 2001).

In Treatment 5 where Thr was included in combination with Trp, an increase in feed intake was noted (946g/d) (the highest feed intake compared to all the treatments in the study) though not statistical significant ($P>0.05$). A study by Edmonds and Baker (1987a) reported

lower feed intakes with piglets when feeding 4% additional Trp and Thr, respectively. These results are typical of an amino acid imbalance in the feed. Harper *et al.* (1970) and Henry (1985) also found a depression in feed intake when there was a deficiency in the limiting amino acid, or an excessive supply of essential amino acids. When an individual amino acid greatly exceeds its requirement in the diet of animals it has a negative impact on the growth and feed intake of the animal (Harper *et al*., 1970).

Trp is involved in the synthesis of serotonin, a neurotransmitter involved in the regulation of feed intake (Henry *et al*. 1992; D'Mello, 2003). It was found by Fernstrom & Wurtman (1972) that the higher feed intake seen in a diet where Trp was added could be due to the Trp: large neutral amino acids (LNAA) ratio. LNAA are neutral in plasma and include tyrosine, phenylalanine, leucine, isoleucine and valine. All these amino acids share a common transport system through the blood-brain barrier and thus compete to be absorbed into the brain (Fernstrom & Wurtman, 1972). Pluske *et al.* (1997) found a positive relationship between feed intake and villi height in the post-weaner piglet. They also speculated that this positive relationship may affect the overall nutrient efficiency of the piglet. In this study where there was an addition of Trp to the diet (Treatment 3) the feed intake increased (887 g/d) compared to the control diet (846 g/d) although this increase was not significant ($P>0.05$). It could well be that when Trp and Thr were added to the basal diet in this study, the amino acid balance improved. The additional Thr could have had a positive effect on the gut and villi development, whereas the additional Trp might have increased serotonin production, all of which contributed to the increase in feed intake. It is also important to note that the basal feed of this experiment had a Trp:Lys of 80, which is 4 times higher than the recommended ratio of about 20. Thus, the added benefit of higher concentrations of Trp in the treatment diets might not have been realised because of the high Trp in the control diet.

In this study the efficiency of feed usage varied between 503 g and 554 g body weight gained/kg feed consumed. When Thr was added to the basal diet (Treatment 2) the feed efficiency decreased significantly (503 g gained/ kg feed consumed) compared to the other treatments.

Excessive amino acids consumed must be deaminated by the body before the nitrogen can be excreted in the form of urea in mammal animals (McDonald *et al*., 2002; D'Mello, 2003). This deamination process is a very energy inefficient process.

5.3 Treatment effects and immune reactions

According to literature there are specific amino acids that have a direct influence on the immune system by improving the cells of the intestine in various ways; they are involved in the hormones that stimulate feed intake and are also a fuel source for immunological cells (D'Mello, 2003; Lalles *et al.*, 2009). Amino acids reported to have an influence on the immune system include threonine (Thr), tryptophan (Trp) and glutamine (Gln) (Lalles *et al.*, 2009). Inclusion of these amino acids in piglet diets at concentrations higher than that required for growth holds potential for reducing post weaning stress.

The titre scores all peaked at day 7, ranging from 10 to 20. The highest titre score during the first week (20) was obtained when additional Thr (Treatment 2) was added to the basal feed. Similar findings were obtained by Li *et al.* (1999); Wang *et al.* (2006) and Cuaron (1984) when they supplemented the feed with Thr.

According to Bowland (1966), Liu & Putman (1979) and Low *et al.* (1979) immunoglobulin contains high amounts of Thr. In studies done by Li *et al.* (1999) and Wang *et al*. (2006) they found that the concentrations of IgG increased when piglets consumed a Thr-enriched diet. This was especially true when the piglets were vaccinated with bovine serum albumin. Several other authors (Cuaron, 1984; Hsu *et al.*, 2001) concluded that additional Thr in the diets of sows increased IgG concentrations not only in the body of the sows but also in their milk. Defa, (1998) conducted an experiment with different dietary intakes of Thr and measured its effect on the growth rate, feed intake and immune reaction. Their findings were similar to the findings of the current study. At a concentration of 6.8 g Thr/kg feed they noticed optimal growth and feed intake. At higher concentrations the body weight gain and feed intake of the pigs decreased but the pigs had a better immune reaction. The improved immune reaction observed in this study could be because more Thr was available for antibody production against the ovine red blood cells present in the piglets.

Chapter 6

Conclusions and recommendations

The aim of the study was to investigate the effects of threonine, tryptophan and glutamine and different combinations of these in weaner diets on the growth and immune function of piglets. During weaning piglets undergo stress that may result in inflammation or infection and growth may be compromised. The role of amino acids in reducing post weaning stress and promoting gut health is therefore an important issue for modern day pig farmers.

A 28 day growth trail was performed and body weight gain, feed intake and FCE recorded. The addition of Thr to the basal diet did show a significant decrease in body weight gain and feed efficiency. Although feed intake and the titre value results did not differ significantly, there were noticeable trends depending on the combination of the amino acids included.

It was noteworthy that the addition of Thr to the basal diet caused a decrease in body weight gain. Based on literature findings the latter could be due to an amino acid imbalance in the diet. When there is an oversupply of an amino acid it must be deaminated. This process uses energy, making the energy unavailable to the body to use for growth. Wang *et al*. (2007) found that an excess of Thr had a negative effect on intestinal protein synthesis, perhaps as a result of its competitive inhibition on the absorption of other indispensable amino acids. This effect might also have contributed to a decrease in growth rate in the pigs that received higher levels of Thr in their diet.

The low feed intake, although statistically not significant, that was noted in the piglets that received the high Thr diet could be because of an amino acid imbalance in the diet, caused by the additional Thr. Several studies noted a decrease in feed intake when additional Thr was included in the diet, not only in pigs but in other animals as well. The addition of Thr also decreased the FCE of the piglets. The lower growth rate together with a constant feed intake yielded a lower FCE.

Titre values were used as indication of immune response. Pigs receiving additional Thr showed higher antibody values against sheep erythrocytes.

In this study, the inclusion of Thr resulted in a decrease in body weight gain and feed intake but an improved immune reaction was observed. It could be argued that based on the results and available literature that the additional Thr was incorporated in the intestine of the piglets. This might have improved gut health and caused the intestine to be a better barrier against toxic substances and antigens. This increase in gut health and villi height had a positive effect on the digestion and absorption of the nutrients consumed. Therefore the H_1 hypotheses for Thr was accepted.

None of the other amino acids or combinations of them has an effect on the performance or development of the immune system. Therefor the H_1 hypotheses for all the other amino acids and combinations of them we rejected.

Recommendations

This was a 28 day growth trail with a relative small number of pigs. Based on the trends observed more studies can be considered to explore the reasons for lower body weight gain and lower feed intakes associated with an improved immune reaction when additional Thr was added. In studies to follow, the number of animals per treatment should be increased. This will increase the amount of data collected per treatment and may lead to the discovery of significant differences between treatments in the data collected. Additional parameters should also be investigated, including the intestinal weight of the piglets, as well as the villi heights in the intestine and the Thr content in the endogenous losses so that the reported effects of supplementary Thr on these variables can be confirmed. Amino acid concentrations of the basal feed must be determined before the start of the trial to avoid high inclusions of test amino acids in the control diets. In this study the control diet's Trp content was four times higher than recommended levels and this could have caused a lack of a positive response to Trp addition to the treatment diets.

It was clear from this study that the influence of protein and amino acids on feed intake is still not fully understood. Control of feed intake is a multiple control system and a better understanding is required on how amino acids influence feed intake as well as the role in various physiological systems in the body (Tome, 2004). It is therefore necessary that further studies will be conducted with a focus on the interaction of amino acids and the response of the immune system to make progress in reducing post weaning stress and mortality in pigs.

References

Abbasi, M.A., Mahdavi, A.H., Samle, A.H. & Jahanlan, R. 2014. Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks. Brazilian Journal of Poultry Sciences. Jan – Mar. 16, 35-44

Ajinomoto animal nutrition. 2002. Threonine requirements in pigs. Benefits of L-threonine supplementation. Ajinomoto Eurolysine information. 26.

Batt, R.A.L. 1980. Influence on Animal growth and development. University Park Press, Baltimore, Maryland.

Ball, R. O. 2002. Definition of amino acid requirements in pigs: partitioning between gut and muscle. Amino acids: meat, milk and more! Improving animal production with reproductive physiology. Québec 2002, 17- 25. Comité organisateur du Congrès CSAS 2002.

Bannink, A., Dijkstra, J., Koopmans, S.J. & Mroz, Z. 2006. Physiology, regulation and multifunctional activity of the gut wall: a rationale for multicompartmental modelling. Nutrition Research Reviews. 19, 227–253.

Bertolo, R. C. 1998. Threonine requirement of neonatal piglets receiving total parenteral nutrition is considerably lower than that of piglets receiving an identical diet intragastrically. Journal of Nutrition. 128, 1752-1759.

Blood, D.C. & Studdert, V.P. 1999. Saunders Comprehensive Veterinary Dictionary. 2nd ed. Elsevier Saunders. Oxford.

Boisen, S., Hvelplund, T. & Weisbjerg, M.R. 2000. Ideal amino acid profiles as a basis for feed protein evaluation. Livestock Production Science. 64, 239-251.

Boudry, G., Peron, V., Le Hue rou-Luron, I., Lalles, JP. & Seve, B. 2004. Weaning induces both transient and long- lasting modifications of absorptive, secretory, and barrier properties of piglet intestine. Journal of Nutrition. 134, 2256–2562.

Bowland, J. 1966. In L. M. Bustad, Swine in biomedical research. USA: Frayn.

Brosnan, J.T. & Brosnan, M.E. 2006. Branched-chain Amino Acids: Enzyme and Substrate Regulation. Journal of Nutrition. 136 (1) 2075-2115.

Burgoon, K.G., Knabe, D.A. & Gregg, E.J. 1992. Digestible tryptophan requirements of starting, growing and finishing pigs. Journal of Animal Science. 70, 2493-2500.

Cabre ra, R.A., Usry, J.L., Arrellano, C., Nogueira, E.T., Kutschenko, M., Moeser, A.J. & Odle, J. 2013. Effects of creep feeding and supplemental glutamine or glutamine plus glutamate (Aminogut) on pre- and post-weaning growth performance and intestinal health of piglets. Journal of Animal Science and Biotechnology. 4, 29-40.

Campbell, J.M., Crenshaw, J.D. & Polo, J. 2013. The biological stress of early weaned piglets. Journal of Animal Science and Biotechnology. 10, 4-19.

Campbell, M.K. & Farrell, S.O. 2003. Biochemistry 4th ed. Thomson Learning, Inc.

Carlstedt, I., Herrmann, A., Karlson, H., Sheehan, J., Fransson, L.A. & Hansson, G.C. 1993. Characterization of different glycosylated domains from the insoluble mucin complex of rat small intestine. Journal of Boilogical Chemistry. 268, 18771 – 18781.

Castagné, V., Maire, J.C. & Gyger, M. 1996. Neurotoxicology and amino acid intake during development: The case of threonine. Pharmacology Biochemistry and Behavior. 55, 653-662.

Castagné, V., Finot, P.A. & Maire, J. C. 1994. Effects of diet- induced hyperthreoninemia. II) Tissue and extracellular amino acid levels in the brain. Livestock Science. 54, 41-48.

Castagné, V., Maire, J.C., Monnoz, D. & Gyger, M. 1995. Effect of threonine on the behavioural development of the rat. Pharmacology. Biochememisty and Behavior. 52, 281-289.

Castell, L.M. 2002. Can Glutamine modify the apparent immune depression observed after prolonged, Exhaustive Exercise? Nutrition 18, 371-375.

Chen, Y. & Guille min, G.J. 2009. Kynurenine pathway metabolites in humans: Disease and healthy states. International Journal of Tryptophan Research. 2, 1-19.

Chung, T.K. & Baker, C.H. 1992. Ideal amino acid pattern for 10-kilogram pigs. Journal of Animal. Science. 70, 3102-3111.

Coffey, R.D. Parker, G.R. & Laure nt, K.M. 2000. Feeding and managing the weanling pig. University of Kentucky, College of Agriculture, ASC-149.

Cole, D.J.A. & Van Lunen, T.A. 1994. Ideal amino acid patterns. In: Amino acids in farm animal nutrition. Ed. D`Mello, J.P.F. CAB International, Wallingford, UK. pp. 99-112.

Colson, V., Orgeur, P., Foury, A. & Morme` de P. 2006. Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses. Applied Animal Behaviour Science. 98, 70–88.

Cooney, R., Owens, E., Jurasinski, C., Gray, K., Vannice, J. & Vary, T. 1994. Interleukin-1 receptor antagonist prevents sepsis- induced inhibition of protein synthesis. American Journal of Physiology. 267, E636- E641.

Center of the Study of Living Standards. University of Tokyo. 2011. A comprehensive approach to life science. Web text book. (2nd ed). University of Tokyo.

Cuaron, J. C., 1984. Effect of lysine and threonine supplementation of sorghum gestation diets on nitrogen balance and plasma constitutes in first litter gilts. Journal of Animal Science 58, 631-637.

Department of Agriculture, Forestry and Fishe ries . 2012. Profile of South African Pork market value chain Pretoria South Africa.

De Blas, C., Garcia, A.I. & Carabaño. R. 2000. Necesidades de Treoninaen animales monogastricos. Barcelona, Spain. XVI Curso de Especialización. Conference Proceeding. FEDNA. 1-24.

Defa, L. C. 1998. Effects of dietary threonine on performance, plasma parameters and immune function of growing pigs. Animal feed Science and Technology. 78, 179-188.

Degussa. 2002. Amino acids in animal nutrition. A compendium of recent reviews and reports. Coral sanivet, Bucharest.

D'Mello, J.P.F. 2003. Amino Acids in Animal Nutrition (2nd ed). CABI Publishing, Wallingford., UK.

Doenhoff, M.J. 2000. The Immune system. Chapter 3 in Breeding for disease resistance in Farm Animals. 2nd edition. Edited by Axford, Bishop, Nicholas & Owen. CABI Publishing, Wallingford., UK.

Domeneghini, C., Di Giancamillo, A., Savoini, G., Paratte, R., Bonte mpo, V. & Dell'Orto, V. 2004. Structural patterns of swine ileal mucosa following L-glutamine and nucleotide administration during the weaning period. A histochemical and histometrical study. Histology and Histopathology. 19, 49–58.

Dong, G.Z., & Pluske, J.R. 2007. The low feed intake in newly-weaned pigs: problems and possible solutions. Asian and Australian Journal of Animal. 20, 440-452.

Edmonds, M.S. & Baker, D.H. 1987a. Amino acid excesses for young pigs: effects of excess Methionine, Tryptophan, Threonine or Leucine. Journal of Animal. Science. 64, 1664-1671.

Edmonds, M.S. & Baker, D.H. 1987b. Comparative effects of individual amino acid excesses when added to a corn-soybean meal diet: effects on growth and dietary choice in the chick. Journal of Animal Science. 65, 699-705.

Edmonds, M.S., Gonyou, H.W. & Baker. D.H. 1987. Effect of excess levels of methionine, tryptophan, arginine, lysine or threonine on growth and dietary choice in the pig. Journal of Animal Science. 65, 179-185.

Food and Agriculture Organization of the United Nations .2012. Statistics division

Farhadi, A., Banan, A., Fields, J. & Keshavaezian, A. 2003. Intestinal barrier: an interface between health and disease. Journal of Gastroenterology and Herpetology. 18, 479-497.

Fernstrom, M.H. & Fernstrom, J.D. 1995. Brain tryptophan concentrations and serotonin synthesis remain responsive to food consumption after the ingestion of sequential meals. American Journal of Clinical Nutrition. Feb, 61 (2) 312- 319.

Fernstrom, J. D. & Wurtman. R.J. 1972. Brain serotonin content: physiological regulation by plasma neutral amino acids. Science. 178, 414.–416.

Flynn, N. & Wu, G. 1997. Glucocorticoids play an important role in mediating the enhanced metabolism of arginine and glutamine in enterocytes of post weaning pigs. Journal of Nutrition. 127, 732–737.

Fuller, M.F. 1994. Amino Acid requirements for maintenance, body protein accretion and reproduction in pigs. In: D'Mello, J. P. F. (ed), Amino Acids in Farm Animal Nutrition (2nd ed). CAB International, pp155-184.

Gatnau, R., Cain, C., Drew, M. & Zimme rman, D. 1995. Mode of action of spray-dried porcine plasma in weanling pigs. Journal of Animal Science. 73 (Suppl. 1) 82.

GenStat Twelfth Edition (Procedure Library Release PL20.1)

Gloaguen, M., Le Floc'h, N. & Van Milgen. J. 2013. Le point sur la couverture des besoins en acides aminés chez le porcelet dans des régimes à basse teneur en protéines. INRA Production. Anim. 26 (3), 277-288.

Gruys, E., Obwolo, M.J. & Toussaint, M.J.M. 1994. Diagnostic significance of the major acute phase proteins in veterinary clinical chemistry: a review. Veterinary Bulletin. 64, 1009 – 1018.

Grimble, R. F. 2006. The effect of sulfur amino acids intake on immune function in humans. Journal of Nutrition. June, 136 (Suppl 6) 1600s – 1665s.

Grimble, R.F. & Grimble, G.K., 1998. Immunonutrition: role of sulfur amino acids, related amino acids, and polyamines. Nutrition. (14) 605-610.

Gruys, E., Toussaint, M.J.M., Landman, W.J.M., Tivapasi, M. Chamanza, R. & Van Veen, L. 1998. Infection, inflammation and stress inhibit growth. Mechanisms and nonspecific assessment of the processes by acute phase proteins. Pages 72–87 in Production Diseases in Farm Animals. 10th Int. Conf. T. Wensing, ed. Wageningen Press.

Guzik, A.C. 2002. Tryptophan requirements and the effects of supplemental tryptophan on growth performance, plasma metabolites, and meat quality in nursery, growing, and finishing pigs. PhD thesis, Louisiana State University.

Hamard, A., Séve, B., & Le Floc'h, N. 2007. Intestinal development and growth performance of early-weaned piglets fed a low-threonine diet. Animal. 1, 1134-1142

Hannah, J.S., Dubey, A.K. & Hansen. B.C. 1990. Postingestional effects of a high-protein diet on the regulation of food intake in monkeys. American Journal of Clinical Nutrition. 52, 320–325.

Harpe r, A.E., Benevenga, N.J. & Wohlheuter, R.M. 1970. Effects of ingestion of disproportionate and amount of amino acids. Physiology Review. 50, 428-558.

Harpe r. A.E. & Peters, J.C. 1989. Protein intake, brain amino acid and serotonin concentrations and protein self-selection. Journal of Nutrition. 119, 677–689.

Heger, J. 2003. Essential to non-essential amino acid ratios. In: D'Mello, J. P. F., (ed), Amino acids in farm animal nutrition. (2nd ed). CAB International, Edinburgh. pp.103-124

Heger, J., Frydrych, Z. & Fronek, P., 1987. The effect of non essential nitrogen on the utilization of dietary protein in growing rat. Journal of Animal Physiology and Animal Nutrition. 57, 130-139.

Henry, Y. 1985. Dietary factors involved in feed intake relation in growing pigs: a review. Livestock. Production. Science. 12, 339 - 354.

Henry, Y., Celleaux, Y. & Seve, B. 1992a. Effects of dietary level of lysine and of level and source of protein on feed intake, growth performance and plasma amino acid pattern in the finishing pig. Journal of Animal Science. 70, 188-195.

Henry, Y,. Seve, B., Colleaux, Y., Ganie r, P., Saligaut, C & Jego, P. 1992b. Interactive effect of dietary levels of tryptophan and protein in voluntary feed intake and growth performance in pigs, in relationship with plasma free amino acids and hypothalamic serotonin. Journal of Animal Science. 70, 1873-1887.

Hoskins, L. 1984. Mucin degradation by enteric bacteria: ecological aspects andimplications for bacterial attachment to gut musosa. In. Boedeker E.C. (ed, *Attachment of organisms to the gut mucosa.* pp. 51-67. CRC press.

Hossner, K.L. 2005. Hormonal regulation of farm animal growth. CABI publishing. Oxfordshire UK.

Hsu, C. B., Cheng, S.P., Hsu, J.C. & Yen, H.T. 2001. Effect of threonine addition to a low protein diet on IgG levels in body fluid of first litter sows and their piglets. Asian-Aus. Journal of Animal Science. 14, 1157-1163.

Jankowski, J., Kubinska, M. & Zdunczyk, Z. 2014. Nutritional and immunomodulatory function of Methionine in poultry diets – a review. Ann. Animal Science. 14 (1), 17-31.

Jansman, A. J., Smink, M.W., Van Leeuwen, P. & Rademacher. M. 2002. Evaluation through literature data of the amount and amino acid composition of basal endogenous crude protein at the terminal ileum of pigs. Animal Feed Science & Technology. 98, 49-60.

Jean, C., Rome, S., Mathe´, V., Huneau, J.F., Aattouri, N., Fromentin, G., Achagiotis, C.L. & Tome, D. 2001 Metabolic evidence for adaptation to a high protein diet in rats. Journal of Nutrition. 131, 91–98.

Johnson, I.R., Ball, R.O., Baracos, V.E. & Field, C.J. 2006. Glutamine supplementation influences immune development in the newly weaned piglet. Developmental and Comparative Immunology. 30, 1191-1202.

Keene, J.C. 2001. Dietary supplements of mixtures of indispensable amino acids lacking threonine. Phenylalanine of histidine increase the activity of hepatic threonine dehydrogenase, phenylalanine hydroxylase or histidine, respectively and prevent growth depression in chicks caused by dietary excesses of threonine, phenylalanine or histidine. Journal of Nurtition and Biochemistry. 12 (5), 274-284.

Kindt, T.J., Goldsby, R.A., & Os borne, B.A. 2007. Kuby Immunology. (6th ed). Freeman and Company. New York. USA.

Kirchgessner, M., Fickler, J. & Roth, F.X. 1995. Influence of proline supply on N retention in young growing pigs. Journal of Animal Physiology and Animal Nutrition. 73, 57-65.

Krehbiel & Matthe W.S. 2003. "Absorption of amino acids and peptides". In Amino Acids in Farm Animal Nutrition. Edited by D'Mello, J. P. F. CAB International, pp 41-70.

Krishnan, L., Sahni, G., Kaur, K.J. & Salunke, D.M. 2008. Role of antibody paratope conformational flexibility in the manifestation of molecular mimicry. Biophysical Journal. 94, 1367–1376.

Lallès, J.P., Bosi, P., Janczyk, P., Koopman, S.J. & Torrallardona, D. 2009. Impact of bioactive substances on the gastrointestinal tract and performance of weaned piglets: a review. Animal, 3 (12), 1625-1643.

Lallès, J.P., Boudry, G., Favie r, C., Le Floc'h, N., Luron, I., Montagne, L., Oswald, I.P., Pié, S., Piel, C., & Sève, B. 2004. Gut function and dysfunction in young pigs: Physiology. Animal Research. 53, 301-316.

Law, G. K., Bertolo, R. F., Adjiri-Awe re, A., Pencharz, P.B. & Ball, R.O. 2007. Adequate oral threonine is critical for mucin production and gut function in neonatal piglets. Animal Journal of Physiology, Gastrointestinal and Liver Physiology. 292, G1293–G1301.

Law, G., Adjiri-Awe re, A., Pencharz, P.B. & Ball, R.O. 2000 Gut: Mucins in Piglets are dependent upon dietary threonine. Advances in Pork production. Proceeding of the 2000 Banff Pork Seminar. 11, 10 (Abstract)

Lawrence, T.L.J. & Fowler, V.R. 2002 Growth of farm animals. (2nd ed). CABI Publishing. UK.

Le Bellego, L., Relandeau, C. & Van Cauwe nberghe, S. 2002. Threonine requirement in pigs - Benefits of L-Threonine supplementation. Ajinomoto Eurolysine. Technical information. 26, 1-23.

LeDividich, J. & Sevè, B. 2000. Effects of underfeeding during the wening period on growth metabolism, and hermonal adjustments in the piglet. Domestic Animal Endocrinology. 19,63

Le Floc'h, N., Melchior, D., Le Bellego, L., Matte, J.J. & Seve, B. 2007. Does sanitary status have an effect on tryptophan requirement for growth of post weaning piglets? Journal of Research of Porcine France. 39, 125-132.

Le Floc'h, N., Melchior, D. & Obled, C. 2004. Modifications of protein and amino acid metabolism during inflammation and immune system activation. Livestock Production Science. 87, 37-45.

Levast, B., Berri, M., Wilson, H.L., Meurens, F. & Salmon, H. 2014. Development of gut immunoglobulins A production in piglet in response to innate and environmental factors. Development and Comparative Immunology. 44, 235-244

Lewis, A.J. 2001. Amino Acids in Swine nitrition. Swine Nutrition 2nd ed. CRC press LLC. pp 131.

Li, P., Yin, Y.L., Li, D.F., Kim, S.W. & Wu, G. 2007. Amino acids and immune function. British Journal of Nutrition. 98, 237–252

Li, X., Li, R.O. & Wu. G. 2010. Composition of amino acids in feed ingredients for animal diets. Amino Acids. 10, 1159-1168

Li, D. Z., Zhao, H., Yang, E., Johnson, W. & Thacker, P.A. 1999. A comparison of the intestinal absorption of amino acids in piglets when provided in free form as a dipeptide. Asian-Australian Journal of Animal Science. 12, 939-943.

Liu, Y.S. & Putman, F. W. 1979. Primary structure of a human IgA1 immunoglobulin. I. Isolation, composition and amino acid sequence of the chymotyptic peptides. Journal of biology and chemistry. 254 (8), 2839-2849.

Low, T. L., Lium Y.S. & Putman, F.W. 1979. Primary structure of a human IgA1 immunoglobulin. II. Isolation, composition and amino acid sequence of the whole alpha1 chain and its cyanogen bromide fragments. Journal of Biology and Chemistry. Apr 25. 254, 2850-2858.

Madras, B.K., Cohen, E.L., Messing, R., Munro, H.N. & Wurtman, R.J. 1974. Relevance of free tryptophan in serum to tissue tryptophan concentrations. Metabolic Clinical Experiment 23 (12), 1107-1116.

Marinkovic, S., Jahreis, G.P., Wong, G.G. & Baumann, H. 1989. IL-6 modulates the synthesis of a specific set of acute phase plasma proteins in vivo. Journal of Immunology. 142, 808-812.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. & Morgan, C.A. 2002. Animal Nutrition (6th ed). London: Pearson Prentice Hill.

Moeser, A.J., Klok, C.V., Ryan, K.A., Wooten, J.G., Little, D., Cook, V.L. & Blikslager, A.T., 2007. Stress signalling pathways activated by weaning mediate intestinal dysfunction in the pig. American Journal Physiology Gastrointestinal Liver Physiology. 292, G173-G181.

Moffett, J.R., Els, T., Espey, M.G., Walter, S.A., Streit, W.J. & Manboodiri, M.A. 1997. Quinolinate immunoreactivity in experimental rat brain tumors in present in macrophages but not in astrocytes. Exp. Neurology. 144 (2), 287-301.

Moffett, J.R. & Namboodiri, M.A. 2003. Tryptophan and the immune response. Immunology and Cell Biology. 81, 247-265.

Moore, S. & Stein, W.H. 1948. Photometric ninhydrin method for use in the chromatography of amino acids. Journal of Biochemistry. 176, 367-388.

Montagne, L., Piel, C. & Lallès, J.P. 2004. Effect of diet on mucin kinetics and composition: nutrition and health implications. Nutrition Review. 62, 105–114.

Mosenthin, R. & Rademacher, M. 2003. Digestible amino acids in diet formulation for pigs. In: D'Mello, J.P.F. (Ed), Amino acids in animal nutrition (2nd Edition), CABI Publishing, Wallingford, UK, pp.169–186.

Muramatsu, K., Odagiri, H., Morishita, S. & Takeuchi. H. 1971. Effect of excess levels of individual amino acids on growth of rats fed casein diets. Journal of Nutrition. 101, 1117-1125.

Nahashon, S.N. & Kilonzo-Nthenge, A.K. 2011. Advances in Soybean and Soybean By-products in Monogastric Nutrition and Health. Soybean and Nutrition. Edited by Hany El-Shemy. InTech. pp125.

National Research Council (NRC). 2012. Nutritional Requirements of Swine, (11th ed). National academy press. Washington DC. USA.

Newsholme, P. 2001. Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection? Journal of Nutrition. 131. (9), 2515 – 2522.

Nicholson, J.W.G., Bush, R.S. & Allen, J.G. 1993. Antibody responses of growing beef cattle fed silage diets with and without selenium supplementation. Canadian Journal of Animal Science. 73, 355-365.

Pack, M., Ficker, J., Rademacher, M., Lemme, A., Mack, S., Hohler, D., Fontaine, J. & Petri, A. 2002. Amino acids in Animal Nutrition: A compendium of recent reviews and reports. Degussa. Coral Sanivet: Bucharest.

Pfefferkorn, E.R. 1984. Interferon gamma blocks the growth of toxoplasma gondii in human fibroblasts by inducing the host cells to degrade tryptophan. Proc. Natl. Acad. Sci. USA. Fed. 81 (3), 908 – 912.

Pierzynowski, S.G., Westrom, B.R., Erlanson-Albertsson, C., Ahren, B., Svendsen, J. & Karlsson, B.W. 1993. Induction of exocrine pancreas maturation at weaning in young developing pigs. Journal of .Pedriatric Gastroenterology and Nutrition. 16, 287-293.

Pluske, J.R., Hampson, D.J. & Williams, I.H., 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livestock Prod uction Science. 51, 215-236.

Pluske, J.R., Pethick, D.W., Hopwood, D. E. & Hampson, D.J. 2002. Nutritional influences on some major enteric bacterial diseases of pigs. Nutrition Research Review. 15, 333-371.

Prunier, A., Heinonen, M. & Quesnel, H. 2010. High physiological demands in intensively raised pigs: impact on health and welfare. Animal. 4 (6), 886-898.

Reeds, P.J., Fjeld, C.R. & Jahoor, F. 1994. Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? Journal of Nutrition. 20, 15-22.

Reiter, R. J., Tan, D.X., Poeggeler, A., Menendez-Pelaez, A., Chen, L.D. & Saarela, S. 1994. Melatonin as a free radical scavenger. Implications for aging and age-related diseases. Animals of the New York Academy Sciences. 719, 1-12.

Ren, M., Liua, X.T., Wanga, X., Zhanga, G.J., Qiaoa, S.Y. & Ze nga, X.F. 2014. Increased levels of standardized ileal digestible threonine attenuate intestinal damage and immune responses in Escherichia coli $K88^{+}$ challenged weaned piglets. Animal Feed Science and Technology. 195, 67-75.

Rezaei, R., Wang, W., Wu, A., Dai, Z., Wang, J. & Wu, G. 2013. Biochemical and physiological bases for utilization of dietary amino acids by young pigs. Journal of Animal Science and Biotechnology. 4 (7), 1-12.

Rhoads, J.M. & Wu, G. 2009. Glutamine, arginine, and leucine signalling in the intestine. Amino acids. 37, 111–122.

Roitt, I., Brostoff, J. & Male, D. 2006. Immunology (7^{th} ed). Mosby international ltd. United Kingdom.

Russell, L.E., Cromwe ll, G.L. & Stahly, T.S. 1983. Tryptophan, Threonine, Isoleucine and Methionine supplementation of a 12% protein, Lysine-Supplemented, corn- soybean meal diet for growing pigs. Journal of Animal Science. 56, 1115-1123.

Ruth, M.R. & Field, C.J. 2013. The immune modifying effects of amino acids on gut-associated lymphoid tissue. Journal of Animal Science and Biotechnology. 4 (27), 1-10.

South African Pork Producers Organization: www.sapork.com

Saavedra, J.M., Palkovits, M., Brownstein, M.J. & Axelrod, J. 1974. Serotonin distribution in the nuclei of rat hypothalamus and preoptic region. Brain Research. 77 (1), 157-165.

Sainio, E.L., Pulkki, K. & Young, S.N. 1996. L-Tryptophan: Biochemical, Nutritional and pharmacological aspects. Amino Acids. 10, 21-47.

Sandberg, F.B., Emmans, G.C. & Kyriazakis, I. 2006. A model for predicting feed intake of growing animals during exposure to pathogens. Journal of Animal Science. 84, 1552-1566.

Sarwar, G., Peace, R.W. & Botting, H.G. 1995. Influence of high dietary threonine on growth and amino acids in blood and tissues of rats. Amino Acids. 8, 69-78.

Schrock, H. & Goldstein, L. 1981. Inter-organ relationships for glutamine metabolism in normal and acidotic rats. American Journal of Physiology. 240, E519-E525.

Schwarcz, R., Bruno, J.P., Muchowski, P.J. & Wu, H. 2012. Kynurinines in the mammalian brain: when physiology meets pathology. Natural Review Neuroscience. 13 (7), 465-477.

Seve, B. 1999. Physiological roles of tryptophan in pig nutrition. In Thryptophan, Serotonin and Melatonin: Basics aspect and applications. Huether. Ed. Kluwer academic/ plemun Publications, New York, NY. pp729-741.

Stein, H. 2007. Feeding the pig's immune system and alternatives to antibiotics. London Swine Conference – Today's Challenges – Tomorrow's Opportunities.

Stoll, B. H. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein- fed piglets. Journal of Nutrition. 128, 606-614.

Takahas hi, K., Ohta, N., & Akiba, Y. 1997. Influence of dietary methionine and cysteine on metabolic responses to immunological stress by Escharichia coli lippopolysaccharide injection, and mitogenic response in broiler chickens. British Journal of Nutrition. 78 (05), 815-821.

Thacker, P.A. 2013. Alternatives to antibiotics as growth promoters for use in swine production: a review. Journal of Animal Science and Biotechnology. 4 (35).

Tinker, K.J. & Gous, R.M. 1986. The effect of a dietary leucine excess on the immune responsiveness and growth of chickens. Suid Afrikaanse Tydskrif van Veekunde. 16 (4), 187-191.

Tlaskalova-Hogenova, H., Mandel, L., Trebichavsky, I., Kovaru, F., Barot, R. & Sterzl, J. 1994. Development of immune responses in early pig ontogeny. Veterinary Immunology and Immunopathology. 43, 135-142.

Tome, D. 2004. Protein, amino acids and the control of food intake. British Journal of Nutrition. 92, S27–30.

Trevisi, P., Melchior, D., Mazzoni, M., Casini, L., De Filippi, S., Minieri, L., Lalatta-Costerbosa, G. & Bosi, P. 2009. A tryptophan-enriched diet improves feed intake and growth performance of susceptible weanling pigs orally challenged with E. coli K88. Journal of Animal Science. 87, 148-156.

Turner, J.R. 2009. Intestinal mucosal barrier function in health and disease. Natural Review Immunology. 9, 799-809.

Vente-Spreeuwenberg, M.A.M. & Beynen, A.C. 2003, Diet- mediated modulation of small intestinal integrity in weaned piglet. In: Pluske, J.R., Le Dividich, J., and Verstegen, M.W.A. (Eds). Weaning the pig: concepts and consequences, Wageningen Academic Publishers. The Netherlands, pp.145-198.

Vincek, C., Saco, K., Kusec, G., Kralik, G., Durkin, I., & Scitovski, R. 2012. Modelling of pig growth by S-Function – least absolute deviation approach for parameter estimation. Archive fur Tierzucht. 55 (4), 364-374.

Virdi, V., Coddens, A., De Buck. S., Millet, S., Goddeeris, B.M., Cox, E., De Greve, H. & Depicker, A. 2013. Orally fed seeds producing designer IgAs protect weaned piglets against enterotoxigenic Escherichia coli infection. PNAS. 110 (29), 11809-11814.

Visser, D. 2014. Modern pig production. Kejafa Knowledge Works. Krugersdorp. South Africa.

Wang, X., Qiao, S., Yin, Y., Yue, L., Wang, Z. & Wu, G. 2007. A deficiency or excess of dietary threonine reduces protein synthesis in jejunum and skeletal muscle of young pigs. Journal of Nutrition. 137, 1442–1446.

Wang, X., Qiao, S.Y., Lui, M. & Ma, Y.X. 2006. Effects of graded levels of true ileal digestible threonine on performance, serum parameters and immune function of 10- 25kg pigs. Animal Feed Science and Technology. 129, 264 – 278.

Wershil, B.K. & Furuta, G.T. 2008. Gastrointestinal mucosal immunity. Journal of Allergy and Clinical Immunology. 121, 380-383.

Wu, G. 2009. Amino Acids: Metabolism, Functions and Nutrition. Amino Acids. 37, 1-17.

Wu, G. 2010. Functional amino Acids in growth, reproduction, and health. Advances in Nutrition. 1, 31-37

Wu, G., Bazer, F.W., Johnson, G.A., Knabe, D.A., Burghardt, R.C., Spence r, T.E., Li, X.L. & Wang, J.J. 2011. Triennial growth symposium: Important roles for l-Glutamine in swine nutrition and production. Journal of Animal Science. 89, 2017-2030.

Wu, G., Knabe, D.A. & Flynn, N.E. 1994. Synthesis of citrulline from glutamine in pig enterocytes. Biochemistry Journal. 299, 115–121.

Wu, G., Meier, S.A. & Knable, D.A. 1996. Dietary glutamine supplementation prevents jejuna atrophy in weaned pigs. Journal of Nutrition. 126, 2578-2584.

Xiong, X., Yang, H.S., Wang, X.C., Hu, C.X., Liu, X. and Wu, Xl. 2015. Effect of low dosage of chito-oligosaccharide supplementation on intestinal morphology, immune response, antioxidant capacity, and barrier function in weaned piglets. Journal of Animal Science. 10, 2014-7851.

Yoo, S.S., Field, C.J. & McBurney, M.I. 1997. Glutamine supplementation maintains intramuscular glutamine concentrations and normalizes lymphocyte function in infected early weaned pigs. Journal of Nutrition. 127, 2253–2259.