EFFECT OF DIET TYPE AND DIETARY CRUDE PROTEIN LEVEL ON THE OPTIMAL TRYPTOPHAN-TO-LYSINE RATIO FOR EARLY WEANED PIGS.

By

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DEDICATION

This thesis is dedicated to my wife and to my mother for helping me to become a better person and a better professional; also I would like to dedicate this work to Chiara, my beautiful daughter and to the Memory of my loving father.

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FOREWORD

This thesis was prepared following a manuscript format. There are three manuscripts corresponding to three chapters. All of the manuscripts have been formatted to meet the guidelines for the Journal of Animal Science manuscript preparation.

ABSTRACT

Depending on dietary factors such as crude protein (CP) or diet type, tryptophan (Trp) could be the second or third limiting amino acid in nursery diets. Two experiments were conducted to evaluate these effects on the Trp-to-lysine ratio (TLR) in early weaned pigs. The first one evaluated the effect of diet type using a combination of two factors: diet type and TLR. The second one tested the effect of CP using two factors – i.e. CP level and TLR. Standardized ileal digestible (SID) lysine (Lys) level was set to 1.31%. Pigs were allowed ad libitum access to feed and water. Average daily feed intake (ADFI), average daily gain (ADG), gain-to-feed ratio (GFR) and plasma urea nitrogen (PUN) were measured weekly for 3-wk. There were no effects ($P > 0.10$) of TLR or the interactions with diet type or CP levels in all of the parameters evaluated. The evidence suggests that the diets had a surplus of Lys hence the lack of effect of the variation in TLR. To further investigate this situation two extra experiments were planned. In the first one, Yorkshire x Landrace weanlings were fed a wheat-barley-Lys-deficient basal diet (0.89% SID Lys) supplemented with crystalline Lys (to create graded levels of SID Lys from 0.89 to 1.33%). Pigs were allowed *ad libitum* access to feed and water. GFR and PUN were measured every 5-d for 15-d; and those parameters were analyzed using the non-linear (NLIN) procedure (broken-line analysis) of SAS to determine the Lys requirement. As SID Lys level increased from 0.89 to 1.33%, the GFR increased (from 0.57 to 0.62 BW gain/kg feed) linearly ($P = 0.03$), PUN decreased (from 5.28 to 3.45 mmol/l) linearly ($P = 0.01$) and quadraticly ($P = 0.08$). The SID Lys requirement for early weaned pigs fed wheat-barley based diets was

estimated to be 1.02%. Since Trp requirements are usually expressed as a ratio to Lys, the objective of the next experiment was to determine the optimal TLR. Pigs were fed a wheat-barley-Trp-deficient basal diet (0.92% SID Lys; 1.7% SID Trp; 19.99% CP) supplemented with crystalline Trp (to create graded levels of SID TLR from 17.75 to 23.61%). Pigs were allowed *ad libitum* access to feed and water. ADFI, ADG, GFR and PUN were measured weekly and used as response criterion. After 3 wks, 3 barrows from each treatment were selected for a nitrogen (N) balance study. Analyzing N retention with the broken-line analysis, the optimal SID TLR lied outside of the dose range evaluated (P =0.02). All the other parameters evaluated failed ($P > 0.10$) to yield an estimate. The reason for the lack of effect may be due to the fact that there was an underestimation of the Trp content of the feed ingredients used, shifting the targeted SID TLR range of the study to higher values (i.e., the proposed values ranged from 13.9 to 21.3%; the analyzed values ranged from 17.8 to 23.6%). Based on the evidence we suggest that the optimal SID TLR for early-weaned pigs fed a wheat-barley diet lies below 17.75%.

Key Words: lysine, tryptophan, requirements, weaned pigs

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LIST OF ABBREVIATIONS

AA	Amino acid(s)
ADFI	Average daily feed intake
ADG	Average daily gain
AID	Apparent ileal digestible
ANF	Anti-nutritional factors
BW	Body weight
CGF	Corn gluten feed
CP	Crude protein
CRD	Complete randomized design
Cys	Cysteine
d	Day(s)
DM	Dry matter
DNA	Deoxyribonucleic acid
GFR	Gain-to-feed ratio
GIP	Glucose-mediated insulin tropic polypeptide
GIT	Gastro intestinal tract
h	Hour(s)
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon gamma
Ile	Isoleucine
Leu	Leucine
LNAA	Large Neutral Amino Acids

Lys	Lysine
ME	Metabolizable energy
Met	Methionine
N	Nitrogen
NIR	Near-infrared
Phe	Phenylalanine
PSE	Pale Soft Exudative Pork
PUN	Plasma urea nitrogen
RBD	Complete randomized block design
SBM	Soybean meal
SDBP	Spray-dried blood plasma
SID	Standardized ileal digestible
TDO	Tryptophan 2,3-dioxygenase
Thr	Threonine
TID	True ileal digestible
TLR	Tryptophan-to-lysine ratio
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
wk	Week(s)

CHAPTER 1

GENERAL INTRODUCTION

Tryptophan (Trp) is an aromatic amino acid (AA) that was first isolated in 1901 by Frederick Hopkins; through hydrolysis of digesta of casein (Hopkins and Cole, 1901). Plants and microorganisms can synthesise Trp from shikimic acid or anthranilate; however, mammals cannot synthesize Trp, hence they require a daily supply of Trp from the diet (Comai et al., 2005).

The importance of Trp for mammals is related to the different roles it plays in the organism. In addition to its main role in protein deposition, it is involved as a precursor on the synthesis of serotonin (Allegri et al., 2003) and to serve as a precursor for the formation of nicotinamide adenine dinucleotide (NAD^+) when the levels of niacin are low (Schröcksnadel et al., 2006).

There are many catabolic routes for Trp. Among those, two pathways are the most important: the kynurenine pathway and the serotonin pathway (Comai et al., 2005). Quantitatively, the major pathway for Trp is the kynurenine pathway which is responsible for over 90% of Trp catabolism. Two enzymes regulate this pathway, a hepatic one, tryptophan 2,3-dioxygenase (TDO; Allegri et al., 2004), producing different metabolites such as picolinic acid, quinolate (which is a precursor for the formation of NAD^+), etc (Moffett and Namboodiri, 2003). The other enzyme (an extrahepatic one) involved in this pathway is indoleamine 2,3-dioxygenase (IDO) which catabolises the formation of kynurenine (Widner et al., 2000).

On the other hand, the serotonin pathway is responsible for catabolising only 1% from the whole Trp ingested (Le Floc'h and Sève, 2007). Trp is transported to the brain where it enters the neuron cells through a transport system named brain-blood-barrier (Purves et al., 2001). The importance of this metabolic route is the production of serotonin by the brain cells, where Trp is hydroxylated and decarboxylated to produce this neurotransmitter linked to sleep, feed intake, behaviour and stress response (Siegel et al., 1999). Another major role of serotonin is acting as a precursor for the formation of melatonin (Purves et al., 2001). Trp has also been associated with the immune response (Mellor and Munn, 1999); macrophages can reduce infections by the production of IDO, which is one of the enzymes of Trp catabolism (Moffett and Namboodiri, 2003).

It has been shown that the synthesis of serotonin is highly dependent on the availability of Trp to the brain (Widner et al., 2000). The passage of Trp to the brain is regulated by the blood-barrier-system. Large neutral AA (LNAA) compete with Trp to utilize this transport (Purves et al., 2001). Many authors suggest that increasing the level of Trp in the diet can lead to an elevation of serotonin levels (Fernstrom, 1986; Henry et al., 1992; Eder et al., 2001; Le Floc'h and Sève, 2007).

In today's swine industry there is a tendency to feed lower crude protein (CP) diets supplemented with AA; in an effort to decrease feeding costs and reduce environmental impact of pig production (Kerr et al., 2003; Shriver et al., 2003; Khendal et al., 2007). It has been shown that inadequate levels of Trp can affect growth performance of pigs by reducing feed intake (Henry et al., 1992). Lys is usually the first limiting AA in swine diets, and Trp is usually considered the

second or third limiting AA (Guzik et al., 2002). There have been several studies in the past 20 years regarding the optimal Trp requirement for pigs, but those studies produced variable results. Those differences could be attributed to genetics, energy content of the diet, CP level and the variability of Trp digestibility among feedstuffs (Burgoon et al., 1992). For weaned pigs, the literature offers dietary requirement that ranges from 0.15% (Zimmerman, 1975) to 0.23% (Sève et al., 1991) of total Trp. In a way to reduce variations on digestibility of feedstuff, the use of digestible Trp (as opposed to total Trp) has been suggested (Guzik et al., 2002); but there are still differences in the recommendations of Trp requirements (Burgoon et al., 1992; Hann et al., 1993).

There is a need to conduct additional research in order to find the optimal TLR in different situations, such as type of diet used, the age and body weight of the pigs and the CP level of the diets. Therefore, the objective of this research was to determine Trp requirements for weaned piglets and how this is affected by dietary composition and dietary protein levels.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Trp is an essential AA that plays major roles in the organism (Schröcksnadel et al., 2006). Quantitatively, the major metabolic route of Trp metabolism is the kynurenine pathway. In this pathway the cofactor NAD^+ is synthesized. Two key enzymes regulate this metabolic route, a hepatic enzyme, Trp 2,3-dioxygenase (TDO) and an extrahepatic one, indoleamine 2,3-dioxygenase (IDO; Allegri et al., 2003). The activity of the kynurenine pathway enzymes vary among species, and among individuals of the same specie due to factors such as health status, genetics or age (Allegri et al., 2004). The second important metabolic route of Trp metabolism is the serotonin pathway, in which serotonin (a neurotransmitter) is synthesized (Siegel et al., 1999). This synthesis is highly depended of the availability of Trp. This pathway is also important because it leads to the formation of melatonin. Trp also plays a key role in different biological functions such as feed intake, behaviour and stress response and immune response (Widner et al., 2000).

In pigs, particularly in weaned piglets, there are diverse recommendations regarding the level of Trp in diets. Those levels of Trp range from 0.13% to 0.24% (total Trp). There are many factors that can explain those differences, including diet type and level of CP as well as many others (i.e. age, digestibility of Trp in feedstuff, health status, etc; Susenbeth and Lucanus, 2005). Usually the

requirement of Trp is expressed relative to lysine (Lys; generally the first limiting AA). Due to the vast differences in the literature about the optimal Trp supplementation, there is still a need of further research in this area, in order to find the optimal TLR for weaned pigs.

2.2 TRYPTOPHAN METABOLISM

Bacteria, fungi and plants have capability to synthesize Trp from phosphoenolpyruvate (Moffet and Namboodiri, 2003). Humans and animals cannot synthesize Trp, hence this AA is essential for them (Comai et al., 2005). Mammals need to degrade external sources of protein in order to meet their Trp requirement.

Trp has several functions in the body. Primarily, Trp is required for the synthesis of proteins, but Trp can act as a precursor of many biological compounds (Allegri et al., 2003; Schröcksnadel et al., 2006). For example, Trp is used for the synthesis of serotonin and melatonin. In addition, when dietary niacin contents are low, Trp is required for the synthesis of NAD^+.

Many problems are associated with the lack of Trp in the diet. It has been reported that Trp deficiency can cause a nitrogen (N) imbalance that could lead to a loss of muscle mass (Widner et al., 2000). In addition, inadequate levels of Trp in the animal's diet could cause a reduction in body weight (BW) gain, feed intake and feed conversion efficiency in chickens (Corzo et al., 2005) and pigs (Cortamira et al., 1991).

In mammals there are several routes through which Trp is metabolized (Comai et al., 2005). The principal (quantitatively) is the kynurenine pathway which leads to the synthesis of niacin (Allegri et al., 2003); hence the importance of this catabolic route, because niacin is a precursor of NAD^+. The second in importance is the serotonin pathway.

2.2.1 Dietary tryptophan utilization

When dietary Trp reaches the liver, one part is used for protein synthesis, another part is degraded through the kynurenine pathway, while the remaining Trp is delivered to extrahepatic tissues where it may also be used for protein synthesis (Moffett and Namboodiri, 2003).

In healthy individuals, the utilization of dietary Trp is as follows (Figure 2.1): 30% is incorporated into proteins; 1-2% is used for synthesis of 5-hydroxy-indoleacetic acid in the serotonin pathway; 3% is used for 3-indoxysulphate synthesis by intestinal bacteria; 3-4% is used for synthesis of 3-indoleacetic acid; an unknown quantity is used for biosynthesis of melanin; 1-2% is excreted as metabolites; and the rest is used for the synthesis of non-aromatic compounds (Allegri et al., 2003).

a) Kynurenine Pathway

Excluding protein synthesis, the kynurenine pathway (Figure 2.2) is the major metabolic route in which Trp is utilized (Allegri et al., 2003; Comai et al., 2005; Mackay et al., 2006; Schröcksnadel et al., 2006). This pathway is also one of the most important routes of the whole organism because it leads to the formation of

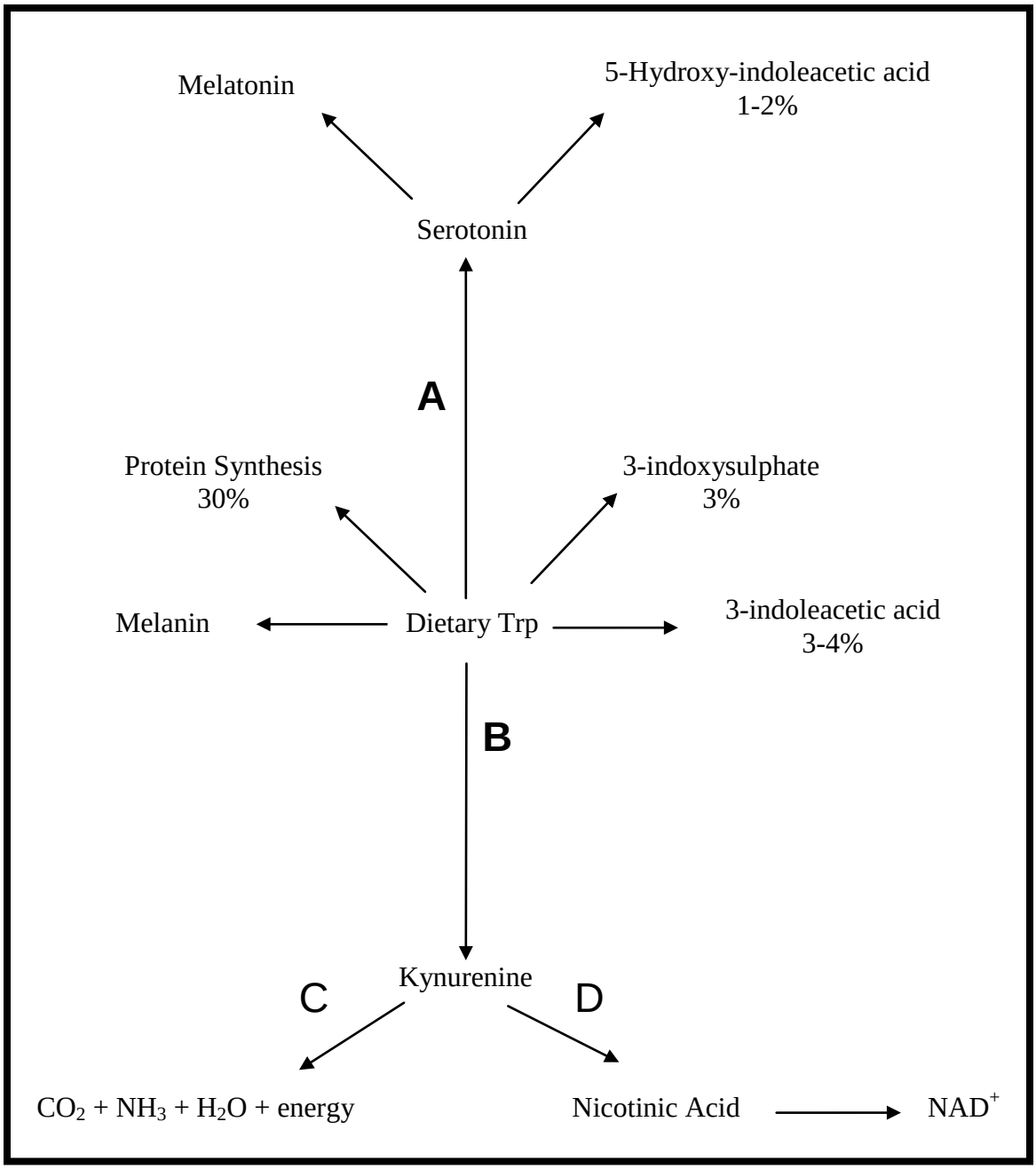

Figure 2.1 Dietary Tryptophan Distributions

(A) Serotonin Pathway. (B) Kynurenine Pathway. (C) Glutarate Pathway. (D) NAD^+ Pathway.

Source: Allegri et al. (2003).

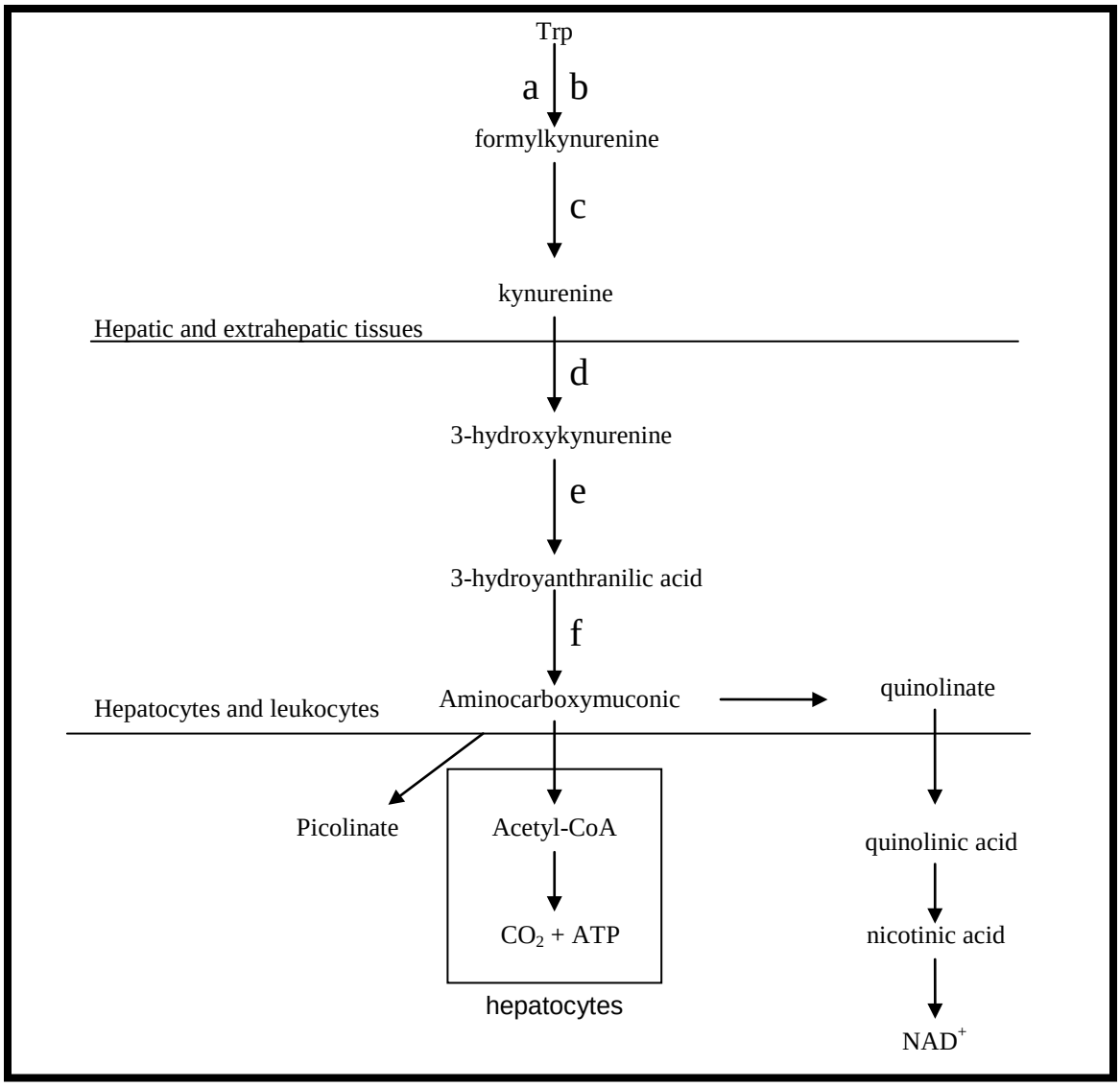

Figure 2.2 Kynurenine Pathway

(a) Trp dioxygenase (TDO). (b) indole amine dioxygenase (IDO). (c) kynurenine formamidase. (d) kynurenine 3-monooxygenase. (e) kynureninase. (f) 3-hydroxyanthranilate 3,4-dioxygenase.

Source: Allegri et al. (2004); Mackay et al. (2006); Moffett and Namboodiri (2003).

NAD^+ (Allegri et al., 2004), which is a cofactor involved in many reactions, including the repair of deoxyribonucleic acid (DNA; Moffett and Namboodiri, 2003).

Trp metabolism begins in the liver by the action of the enzyme TDO (Allegri et al., 2004). TDO degrades Trp to kynurenine (Le Floc'h et al., 2004). One part of the kynurenine produced is metabolized to kynurenic acid and anthranilic acid. The other part, is metabolized to 3-hydroxykynurenine (Wolf, 1974). 3-hydroxykynurenine may be degraded to quinolate, which is a precursor for the formation of NAD^+ (Satyanarayana and Rao, 1980); or may be metabolized into amino carboxymuconic semialdehyde, which can be decarboxylazed to picolinate (Allegri et al., 2004). Extrahepatic tissues may also metabolize Trp by IDO, producing kynurenine (Allegri et al, 2004). It has been suggested that IDO is induced by an inflammatory cytokine, interferon-γ (IFN-γ) in many extrahepatic cells (Widner et al., 2000).

The activity of the enzymes of the kynurenine pathway varies under specific conditions such as health status, type of diet, age, genetics, etc. Allegri et al. (2004) studied the impact of hypercholesterolemia on the kynurenine pathway by evaluating two breeds of rabbits (New Zealand that acted as the control, and Watnabe Heritable Hyperlipidemic -WHHL) and two diets (cholesterol free and cholesterol/high lipid diet). The WHHL breed is known for their spontaneous development of hyperlipidemia and atherosclerosis. Results of the study indicated a high fat diet and hereditable hyperlipidemia could cause a decrease in TDO activity; however, no effects were observed in IDO activity. In addition, it has been reported

that younger individuals of any species have higher activity of IDO, due to a higher synthesis of nicotinic acid, resulting in an energy increment (Comai et al., 2005). Moreover, inflammatory processes, as a result of a disease, can also increase the activity of IDO and TDO (Mackay et al., 2006). For example, Melchior et al. (2004) suggested a decrease of plasma Trp concentration after a chronic lung inflammation in pigs. This could be due to a high activity of IDO induced by IFN-γ.

b) Serotonin Pathway

The other metabolic route of Trp catabolism is the serotonin pathway (Figure 2.3). Approximately 1% of the total Trp ingested is converted to serotonin.

The first step of this metabolic route is the transportation of Trp from the blood to the brain. Then, Trp together with LNAA, such as tyrosine (Tyr), phenylalanine (Phe), Leucine (Leu), Isoleucine (Ile) or Valine (Val) enter the neurons cells (through the brain-blood-barrier) via plasma membrane transporters (Purves et al., 2001). In the neurons, Trp is hydroxylated to 5-hydroxytryptophan (5-HTP) by Trp hydroxylase. This enzyme requires oxygen and a cofactor (BH4) to be activated. Then, 5-HTP is decarboxylated to serotonin by the action of the aromatic L-amino acid decarboxylase enzyme (Siegel et al., 1999). The hydroxylation of Trp seems to be the limiting step in the synthesis of serotonin due to the fact that 5-HTP is found only in trace amounts in the brain, suggesting that it is quickly converted to serotonin (Siegel et al., 1999).

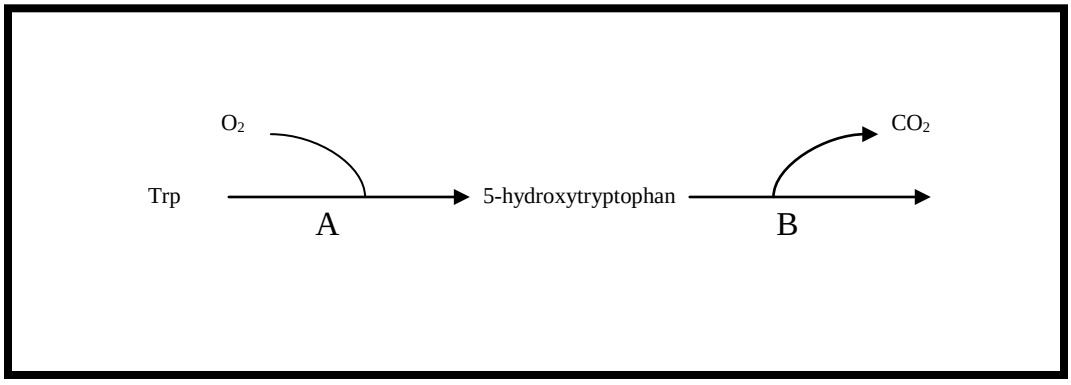

Figure 2.3 Serotonin Pathway

(A) Trp hydroxylase. (B) Aromatic L-amino acid decarboxylase.

Source: Purves et al. (2001).

Serotonin is a neurotransmitter and is very important for the organism. It seems to be involved in every type of human and animal behaviour, circadian rhythmicity, neuroendocrine and brain function. Abnormalities in serotonin function are thought to be related to depression and schizophrenia (Siegel et al., 1999).

The synthesis of serotonin is highly dependent on the availability of Trp (Widner et al., 2000). It has been suggested that when there is a Trp reduction due to chronic stimulation of the immune system with a high IFN-γ production, the availability of Trp for serotonin synthesis is reduced. Le Floc'h et al. (2004) mentioned that during an inflammatory process, cytokines such as IFN-γ, favour tissues involved in the immune response for AA utilization. Furthermore, IDO can use serotonin as a substrate, thus decreasing also serotonin concentration (Widner et al., 2000). In addition, Widner et al. (2000) suggested that psychiatric disorders after chronic immune activation could be related to an abnormal Trp metabolism initiated by IDO. The synthesis of serotonin could be increased under specific

conditions, as a result of the increase rate of conversion from Trp to 5-HTP (Siegel et al., 1999). Requirements for short-term increase of serotonin could be achieved by changing the kinetic properties of Trp hydroxylase, without synthesis of new molecules of the enzyme. In situations where there is a long-term increased requirement, there is need for the synthesis of new molecules (Siegel et al., 1999).

Serotonin is also important because it is a precursor of melatonin. Melatonin is a hormone secreted by the pineal gland. It has many functions on mammals, including as hormone regulator, maintaining the circadian rhythm, assisting in the regulation of release of female reproductive hormones (Reiter, 2003). Melatonin is also linked to a reduction of stress levels and aggressive behaviour in mammals (Kerr et al., 2002). In the brain, serotonin is converted to N-acetylserotonin (NAS) in a reaction catabolised by N-acetyltransferase (NAT). NAS is then converted to melatonin by means of the enzyme hydroxyindole-O-methyltransferase (Purves et al., 2001). The synthesis of melatonin is regulated by light and dark cycles during the day. During day-light hours there is a reduction of its synthesis; however during the dark hours melatonin synthesis can be increased up to 10 times (Lepage et al., 2005).

2.3 EFFECTS OF TRYPTOPHAN ON SOME BIOLOGICAL FUNCTIONS

In addition to its main role as an AA, i.e. being part of protein synthesis and build up, Trp is the precursor of two very important compounds. The first one is NAD^+, a cofactor associated with many reactions in the body (Allegri et al., 2003; Comai et al., 2005; Mackay et al., 2006; Schröcksnadel et al., 2006). The second

one is serotonin, a neurotransmitter (Purves et al., 2001). Furthermore, the importance of Trp is also associated with its effect over different biological functions such as feed intake, behavior and stress response, and immune response (Le Floc'h et al., 2002).

2.3.1 Feed Intake

There is evidence suggesting that Trp deficiencies in diets have an adverse effect on appetite (Eder et al., 2001). Serotonin (produced on the brain by hydroxylation of Trp) plays a key role on feed consumption and Trp deficiency can lessen feed consumption due to depletion on serotonin levels (Henry et al., 1992). The negative effects of Trp-deficient diets on feed intake can be increased with low-Trp-protein-rich diets due to the high amounts of LNAA which compete with Trp for transport across the blood-brain-barrier (Le Floc'h and Sève, 2007). Having less Trp available for the brain can decrease serotonin synthesis and affect feed intake (Henry et al., 1992). There is direct relationship between the rate of serotonin synthesis and the supply of Trp to the brain (Fernstrom, 1986). It has also been pointed out that females are more sensitive to the disparity of LNAA than intact males or barrows (Henry et al., 1996).

A reverse effect occurs with rich-carbohydrate diets which can enhance Trp content in the brain via an increase on the insulin secretion, which in turns will increase the ratio of Trp to LNAA (by favoring the uptake of LNAA into the muscle hence reducing their concentration in the blood) causing an increase of the uptake of Trp by the blood-brain-barrier (Maher et al., 1984).

There is also evidence that Trp can boost feed intake by increasing the levels of ghrelin in the gastro intestinal tract (GIT) membrane (Zhang et al., 2007). Ghrelin is a hormone involved in the regulation of feed intake and the signaling from the digestive system to the brain, which stimulates appetite and regulates the release of growth hormone by the pituitary (Kojima et al., 1999). It is produced in the GIT and regulates glucose and energy balance (Wiedmer et al., 2007).

There is evidence that feed selection can also be affected by the levels of Trp in the diet. In a study done by Ettle and Roth (2004) comparing two piglet diets (a non-deficient versus a Trp-deficient diet); they found that piglets preferred the non-deficient diet suggesting that pigs posses some sensory recognition for Trp.

Finally, there is evidence that Trp can also increase gastric emptying. Porter et al. (1994) found an increase on plasma insulin concentration in early weaned pigs (fed a low-Trp diet for 22 d) after a glucose and Trp load. This was explained to be due to an increase in plasma glucose-mediated insulin tropic polypeptide (GIP; Cortamira et al., 1991).

2.3.2 Behaviour and Stress Response

Pigs have to endure different stressful situations along their production live such as weaning, mixing and remixing, and relocation; the augment on stress levels being usually associated with a decrease on animal performance (Adeola and Ball, 1992). There is evidence that dietary Trp can help to reduce the levels of stress of those situations (Sève et al., 1991; Koopmans et al., 2005). This effect of Trp is related to its role in the synthesis of serotonin. This neurotransmitter has the

capability to reduce hostile behavior and to lessen the levels of stress (Li et al., 2006).

There is some contradictory evidence about the effect of dietary Trp on stress response and behavior modification. In a study done by Sève et al. (1991), comparing emotional vs. non-emotional pigs (this differentiation was based on their behavioral reactivity to a stress situation done by a parallel study, Meunier-Salaün et al., 1991). The authors found that behavioral reactivity (measured on three categories: vocalization, ambulation and exploration time) (Meunier-Salaün et al., 1991) was not affected by the increase of Trp in the diet. However more recent studies point to a positive effect of dietary Trp on reduction of stress levels (Bowker et al., 2000; Peeters et al., 2004; Koopmans et al., 2006).

Of particular importance is the stress caused to pigs during transportation, especially to the abattoir. It has been pointed out that stress can be one of the causes of pale soft exudative pork (PSE; a condition that affects pork quality). Pigs with higher susceptibility to stress present an increased concentration of lactic acid, elevation of muscle temperature and an increase on muscle stiffness favoring the incidence of PSE (Bowker et al., 2000). Adeola and Ball (1992) found a reduction on the incidence of PSE when Trp (0.5%) and Tyr (0.1%) were added to a 14.4% CP diet during 5-d prior to the slaughter. The authors argue that the reduction of PSE was due to the lesser susceptibility to stress of the supplemented pigs compared to the control. This reduced stress level was induced by the higher concentration of serotonin that was caused by the increase of the proportion of Trp and Tyr relative to LNAA. In another study, Peeters et al. (2004) found that the

addition of Trp had a calming effect on the animals, translated into an increase of the time that pigs spent lying down during and after transport (i.e. less time available for fighting with other pigs).

Another stressful situation on the pig life is weaning because piglets experience physiological, nutritional, environmental and social changes (piglets are relocated to the nursery barn), fights and stress arose among the weaned pigs. In North America, the age of weaning is around 3 to 4 weeks (Ensminger and Parker, 1997; Robert et al., 1999). Immediately after weaning the piglets are more susceptible to some infectious agents (e.g. *E. coli* and *Mycoplama*) and have a depressed growth rate during these first few days (Robert et al., 1999; Ruis et al., 2000). These situations have a negative effect on the weaned piglet performance. Koopmans et al. (2006) found a decrease on physical activity (i.e. fighting with pen mates) only after 10 d of Trp supplementation. The authors argued that the lack of a quicker response of Trp supplementation was due to a decrease on feed intake at the time of measurement (It was performed 5 d after weaning and mixing. During this period it is not unusual to find a depression on feed intake). The authors also suggested that an increase in the ratio of Trp to LNAA can reduce the levels of cortisol (stress hormone). Indeed, a study from the same research group showed that a 0.5% Trp supplementation for 6 d after weaning can increase the production of serotonin, decreasing salivary cortisol (Koopmans et al., 2006).

There is evidence that dietary Trp can reduce levels of stress. The explanation for this is that after a meal, free Trp (supplemented as crystal AA) enters the brain rapidly and it is rapidly converted into serotonin (short term effect)

while on the other hand, Trp bound to protein takes more time to be released and enter the brain, having a long term effect on serotonin metabolism (Séve, 1999).

2.3.3 Immune Response

Trp also plays a major role in the immune system. Mellor and Munn (1999) indicated that macrophages could reduce infections caused by microbial agents by increasing the production of IDO. IDO is one of the enzymes of the principal pathway of Trp metabolism (kynurenine pathway). During an inflammatory process the cytokine IFN-γ induces the activation of IDO in a way to decrease pathogen infection. There is evidence that some metabolites of this pathway (quinolinate, picolinate) regulate the immune response. In addition, there is also evidence that Trp catabolism is associated with inflammatory reactions of several diseases (Moffett and Namboodiri, 2003).

There is evidence that Trp depletion occurs during the immune response. In addition, some metabolites that participate on the kynurenine pathways have a high activity during this reaction (Schröcksnadel et at., 2006). A decrease of Trp as a result of the activation of IDO (via IFN-γ) reduces the local supply of Trp to parasites, cancer cells or pathogens thus inhibiting growth and proliferation. This theory is known as the "Trp depletion theory" (Mellor and Munn, 1999). In concordance, Widner et al. (2000) suggested that the depletion of Trp during the immune response will decrease the protein synthesis, inhibiting growth of viruses.

Although this theory has been accepted, there are still some issues that have not been clarified. To start with, bacteria can produce their own Trp (Moffett and

Namboodiri, 2003); however this Trp synthesis requires a lot of energy and is easier for the bacteria to use Trp from the external media instead of synthesizing it (Mellor and Munn, 1999). Secondly, there is usually necrosis and apoptosis on the inflammatory sites, causing proteolysis and AA release (including Trp). Furthermore, many pathogens can increase their Trp synthesis (up-regulating gene expression) when they sense a reduction in Trp availability. In addition, this theory is not supported by *in vivo* data (Moffett and Namboodiri, 2003).

Moffett and Namboodiri (2003) proposed an alternative to the depletion theory: "the utilization theory". This theory is based on two hypotheses. The first one indicates that the metabolites of the kynurenine pathway (such as quinolinate, picolinate, etc.) act as regulators of the immune response. The second one assumes that quinolinate is stored in specific immune cells for extrahepatic NAD^+ synthesis to prevent depletion of this cofactor as a result of DNA damage caused by the immune response (i.e. the generation of N and oxygen species by phagocytes). However, Moffett and Namboodiri (2003) mentioned that modulation of the immune response needs both Trp depletion and metabolite production.

Mellor and Munn (1999) suggested that IDO plays a crucial role in immune regulation. IDO could decrease T-cell proliferation and help them to differentiate between foreign and self antigens. IDO activates kynurenine pathway metabolism in mature denditric cells. Therefore, these cells could catabolise Trp. On the other hand, the immature denditric cells mediate tolerance to self antigens and inoffensive foreign antigens (Moffett and Namboodiri, 2003). Widner et al. (2000) suggested

that the Trp depletion could reduce T-cell proliferation and thus extended tissue damage could be prevented.

There is evidence that some metabolites of the kynurenine pathway could be neurotoxic. For example, quinolinate has been implicated in many neurodegenerative disorders such as Alzheimer's disease and AIDS dementia complex (Moffett and Namboodiri, 2003). Widner et al. (2000) suggested that the "excitotoxic capacity of quinolinate could favour the development of neuronal dysfunction and nerve cell death". In the inflammatory process, the cytokine IFN-γ induces the activation of IDO in a way to decrease pathogen infection.

2.4 TRYPTOPHAN REQUIREMENT OF NURSERY PIGS

In the past decades there have been several studies on the optimum concentration of Trp in diets for nursery pigs. However, there are some differences among these recommendations (Susenbeth and Lucanus, 2005). For example, the official German Recommendation (GFE, 1987) states a Trp requirement of 20% of the total Lys requirement while the National Research Council (NRC, 1998) recommends 17.78% and 18.26% for body weight from 5 to10 kg and 10 to 20 kg, respectively, and the Agricultural Research Council (ARC, 1988) estimates the requirement of Trp at 15% (Eder et. al., 2003).

These differences can be explained by factors such as CP level, different kind of feedstuffs, and the variability Trp digestibility in different feedstuffs. Indeed, some researches have used the true ileal digestible (TID) Trp in order to avoid the effect of feedstuffs variability (Guzik et al., 2002). These authors in their research,

found values for the TID Trp requirement for nursery pigs of 0.21% (17.14% TLR), 0.20% (18.11% TLR) and 0.18% (19.82% TLR) for phase I (5.2 to 7.3 kg), II (6.3 to 10.2 kg) and III (10.3 to 15.7 kg), respectively. These values are slightly similar to those suggested by the NRC (1998), 0.22% of TID for 5 to 10 kg and 0.18% for 10 to 20 kg. However, there are still some differences on these values too. For example, Burgoon et al. (1992) found a value of 0.15% (TID Trp) for starting pigs (6 to 16 kg). Han et al. (1993) recommends supplementation levels of 0.16% (TID) for pigs in the body weight range of 10 to 20 kg.

Depending on the components of the diet, Trp could be limiting in nursery pigs (Guzik et al., 2005). According to Guzik et al. (2002) Trp is equally second or third limiting in diets for pigs. Sato et al. (1987) mentioned that Trp is the first or second limiting AA in corn, corn-fish meal, corn-meat-bone meal and low-protein corn-soybean meal (SBM) diets for pigs. This suggestion is supported by Guzik et al. (2002), who mentions that Trp is the first limiting AA in corn diets. Russell et al. (1987) suggested that a corn-SBM diet, supplemented with Lys, Trp and Thr is not limiting in Met and Cys or N, but Val could be the only limiting AA.

Today the swine industry is focusing more on the ideal protein concept (ARC, 1981). This concept states that the entire essential AA are limiting for performance, and the ratio of these AA to the first limiting AA (i.e. Lys) is constant. Lys was chosen because it is usually the first limiting AA in swine diets and because it is mainly used for protein deposition. So to calculate the requirement of any essential AA (regardless of age, BW, breed, etc) we just need to calculate the requirement of Lys, and then use its ratio to Lys to calculate the desired AA requirement (Boisen,

2000). The ideal protein ratios can be found in the NRC (1998) recommendations. For these reasons, Trp requirements are often expressed as a ratio to the AA that becomes limiting when Trp values reach requirement, usually Lys (Susenbeth and Lucanus, 2005) rather than percentage of dietary intake or amount required per day. Indeed, Susenbeth (2006) could not find an optimal Trp recommendation in experiments expressing Trp requirement as concentration or daily intake. Susenbeth and Lucanus (2005) suggested that Trp supplementation expressed as concentration or amount of daily intake are strongly dependent on Lys levels, hence it should be expressed as a ratio to Lys.

Providing an accurate requirement of Trp has proven difficult due to many factors such as age, weight or protein content of the diet (Guzik et al., 2002). The importance of the diet type on the TLR was demonstrated by Guzik et al. (2005) who found some differences on the TLR between corn diets and wheat-barley diets. They suggested an increase over the NRC (1998) recommendations when the diet is made up without corn. The NRC (1998) estimates it at 0.18% for pigs from 5 to 20 kg.

2.4.1 Effect of crude protein on tryptophan requirement

In today's swine industry there is a tendency to use low-protein diets supplemented with crystalline AA to reduce feeding cost and, in addition to decreasing environmental impact of swine facilities due to a reduction in N excretion (Shriver et al., 2003; Khendal et al., 2007). Indeed, reducing CP in pig diets reduces N excretion and ammonia emissions from slurry (Shriver et al., 2003).

Kerr and Easter (1995) found that for each percentage unit reduction and with adequate AA supplementation, the total N losses can be reduced by 8% and ammonia gas emission can also be reduced by 20% (Knabe, 1996; Kerr and Easter (1995) suggested using diets with a 12% level of CP supplemented with Lys, Trp and Thr to maximize N retention.

Understanding the effect of CP on AA metabolism, especially Trp, will be useful when formulating these low CP diets. Reducing CP levels of the diet without adequate AA supplementation could decrease growth performance of pigs (Kerr et al., 1995). The authors concluded that the low performance of pigs, as consequence of low protein diets, could be improved by supplying appropriate AA to the diets. This affirmation is also supported by Le Bellego and Noblet (2002) who found that a reduction of 5% in CP supplemented with Lys, Thr, Met, Trp and Val had no negative effect on growth performance and conformation of piglets.

Requirement of essential AA (expressed as percentage of diet) in monogastrics increase when dietary CP level is increased (Strieker et al., 2006). An early study with broiler chicks by Grau and Kamei (1950) found that an increase in CP also increased the requirement of Lys and Met plus Cys. Later, Griminger et al. (1956) found that an increase in CP also increased the Trp requirement in chicks. It has been pointed out that increasing those AA without increasing the limiting AA (i.e. Trp) can lead to a reduction in performance (Boomgaardt and Baker, 1973); the authors estimated the minimal requirements of Trp for maximal weight gain for nursery pigs fed a corn-gelatin diet. However, these authors found a decrease on

Trp requirement when CP level was increased. They found that the requirements of Trp were 0.71%, 0.67% and 0.66% of the CP at 10%, 14% and 18% of CP.

. However, the AA requirement expressed as percentage of CP is constant when the CP level changes from suboptimal to optimal levels (Boomgaardt and Baker, 1973). Hurwitz et al. (1998) found that dietary CP level affected Lys requirements only at levels below optimal. They also found no effect of dietary CP level on AA requirement when those levels were above optimal.

Since Trp competes with LNAA for the transport through the brain-blood-barrier (Le Floc'h and Sève, 2007), diets with high CP content (i.e. with a low Trp:LNAA ratio) may reduce the availability of Trp for serotonin synthesis and at the end reduce feed intake (Henry et al., 1996). As mentioned before, the level of CP could affect the Trp utilization (Guzik et al., 2002).

2.4.2 Effect of diet composition on tryptophan requirement

There is considerable variation in Trp content among feedstuffs for example, cereal grains have a lower content of Trp (Sauer and Ozimek, 1986) with values ranging from 0.11% for barley to 0.20% for corn. On the other hand, SBM has 0.60% and fish meal has 0.66% of Trp (NRC, 1998). In addition, there is also great variation in the digestibility values of Trp. Usually cereal grains have lower apparent ileal digestible (AID) Trp, with values ranging from 64% for rice and corn to 81% for wheat (NRC, 1998). Trp content of feed ingredients and variability in digestibility of Trp among feedstuffs can be one of the factors that explain the differences in Trp requirements.

These variations in AID are due to little changes in endogenous AA losses and to the low content of Trp in these grains (Sauer and Ozimek, 1986). The presence of fiber and anti-nutritional factors (ANF; such as tannins, trypsin inhibitors, etc) can also be accounted for these differences in AID (Nyachoti et al., 1997; Mariscal-Landín et al., 2002).

It has been demonstrated that dietary fiber increases endogenous AA losses in the gut of monogastric animals by sloughing of the epithelia cells or by adsorbing peptides and AA and digestive enzymes (Bergner et al., 1981; Shah et al., 1982). Furthermore, dietary fibre has also been reported to increase digestive secretions and the synthesis of microbial protein (Schneeman et al., 1982; Laplace et al., 1989). In addition, dietary fibre can obstruct the digestion of peptides and AA by proteolytic enzymes, reducing their adsorption by the intestine (Sauer and Ozimek, 1986).

The presence of ANF such as trypsin inhibitors, lectins and tannins can increase endogenous losses by reducing digestion of dietary CP, or by increasing N secretions into the gut lumen (Nyachoti et al., 1997; Myrie et al., 2008). Tannins are naturally present in sorghum, rapeseed, barley and legume seeds; providing resistance to the seed against birds, fungi and pests (Salunkhe et al. 1990). However, when tannins become part of animal feeds, they might reduce AID of AA and CP by binding with dietary proteins and proteolytic enzymes to reduce adsorption of AA in monogastric animals (Jasman, 1993). In a similar manner, trypsin inhibitors increased the production of digestive enzyme from the pancreas, causing pancreatic hypertrophy; resulting in an increase in the endogenous losses of

AA and a reduction of proteolysis (Clarke and Wiseman, 2005). Lectins can also reduce AID of CP and AA by binding with receptors in the small intestine and reducing digestion and utilization of CP and AA (Van Nevel et al., 1998).

Elimination of the ANF can increase apparent digestibility values. For example, Sohn et al. (1994) found higher AID values of AA with isolated soy protein than with whole SBM. The authors suggested that the increase in AID was a consequence of the lower content of ANF of the isolated product.

The literature shows a vast range of optimal TLR, especially for nursery pigs. Many factors have a fundamental role as the source of this diversity. Among those factors, dietary type and CP level are considered two of the most important. Therefore, the objectives of this thesis were to determine the Trp requirement (expressed as a ratio to standard ileal digestible Lys) of early-weaned pigs, and how this ratio is affected by dietary CP level and diet composition.

CHAPTER 3

MANUSCRIPT ONE

Effect of diet type and crude protein level on the optimal tryptophan-to-lysine ratio in early weaned pigs.

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3.1 ABSTRACT

Depending on dietary factors such as crude protein (CP) level or diet type, tryptophan (Trp) could be the second or third limiting amino acid in nursery diets (lysine (Lys) is usually the first one). Two experiments were conducted to evaluate these effects on the standardized ileal digestible (SID) Trp-to-Lys ratio (TLR) in early weaned pigs. In Exp. 1, 96 Yorkshire x Landrace x Duroc pigs (n=96, initial body weight (BW): 7.23 ± 0.19 kg) were blocked by BW, group-housed, and allotted to 4 dietary treatments (a combination of two factors; 1) diet type: Corn-SBM or Corn-Wheat-Barley-Peas-SBM; and 2) SID TLR: 15% or 19%) in a 2 x 2 complete randomized design (CRD). In Exp. 2, 96 Cotswold pigs (n=96, initial BW: 6.90 ± 0.28 kg) were blocked by BW, group-housed, and allotted to 4 dietary treatments: a corn-SBM diets with a combination of two levels of CP (20% and 17%) and two levels of SID TLR (20% and 16%) in a 2 x 2 complete blocked randomized design (RBD). In both experiments SID Lys level was set to 1.31% on average; pigs were allowed ad libitum access to feed and water. Average daily feed intake (ADFI), average daily gain (ADG), gain-to-feed ratio (GFR) and plasma urea nitrogen (PUN) were measured weekly for 3 wk. After the performance trial, 3 (experiment 1) or 4 (experiment 2) barrows were selected for a 7-d nitrogen (N) balance study (3-d of adaptation followed by 4-d of total collection of faeces and urine). The Corn-SBM diet had higher ($P < 0.10$) ADG (0.39 kg/d), GFR (0.71 kg BW/ kg feed) and final BW (15.36 kg) than the Corn-Wheat-Barley-Peas-SBM diet (0.36 kg/d, 0.67 kg BW/kg feed, and 14.80 kg). In addition, the Corn-SBM diet had lower ($P < 0.10$) average PUN than the Corn-Wheat-Barley-Peas-SBM diet (1.79

vs. 2.05 mmol/l). The 20% CP diets had higher ($P < 0.01$) ADG (466.70 g/d vs. 420.36 g/d), better ($P < 0.01$) GFR (0.90 vs. 0.81 kg BW/kg feed) and higher ($P < 0.01$) average PUN (1.74 vs. 1.36) than the 17% CP diets. The 20% CP diets consumed (22.82 vs. 20.76 g/d) and excreted (6.41 vs. 4.93 g/d) more ($P < 0.10$) N than the 17% CP diets. There were no effects ($P > 0.10$) of the TLR or the interactions with diet type or CP levels in all of the parameters evaluated; however the only exception was the low TLR diet which had a lower ($P < 0.10$) final PUN (1.68 ± 0.19 mmol/l) than the high TLR diet (1.94 ± 0.45 mmol/l). The evidence suggests that the diets had a surplus of Lys hence the lack of effect of the variation of TLR. Further research is needed to determine the true effect of diet type and CP level on Trp supplementation.

Key Words: crude protein, diet type, pig, tryptophan-to-lysine ratio

3.2 INTRODUCTION

Trp is an essential AA that could be limiting in swine diets (Guzik et al., 2005). It can be equally the first (in a corn-fish meal diet; Sato et al., 1987), the second or the third limiting AA (Guzik et al., 2002). With today's focus on the ideal protein concept (NRC, 1998) an inadequate supply of Trp will affect pig performance (Henry et al., 1992).

Trp requirements for pigs have been studied in the past decades including the requirements for nursery pigs (Susenbeth and Lucanus, 2005). However the total Trp recommended levels for nursery pigs range from 0.13% (Zimmerman, 1975) to

0.23% (Sève et al., 1991). CP level, diverse feedstuff used and the variability of digestibility of Trp on those feedstuffs can be some of the reasons for those variations (Guzik et al., 2002). In an effort to avoid the difference on Trp digestibility, some authors suggest the use of true ileal digestible (TID) Trp (Guzik et al., 2002). However there are still differences on the optimal TID Trp recommendations for nursery pigs (Burgoon et al., 1992; Han et al., 1993; NRC, 1998). Trp requirements are dependant on the level of Lys in the diet; therefore Trp requirements should be expressed as a ratio to Lys rather than a concentration in the diet (Susenbeth and Lucanus, 2005). Providing an optimal TLR requirement has proven difficult due to factors such as CP level and diet type. Guzik et al. (2005) found differences on the TLR on corn diets from wheat-barley diets.

There is a need to conduct additional research in order to evaluate the effect of diet type and CP level on the optimal TLR. Hence, the objectives of this study were to evaluate the effect of diet type (Experiment 1) and CP level (Experiment 2) in the optimal TLR in early weaned pigs using ADFI, ADG, GFR and PUN as response criteria.

3.3 MATERIALS AND METHODS

3.3.1 Experiment 1

Timeline

Pigs arrived at the T.K. Cheung Center for Animal Research (TKCCAR) on July 6, 2006. After 5 d of adaptation, 4 spare pigs (2 barrows and 2 gilts) were removed based on their BW (the heaviest and lightest from each gender); the

performance trial started on July 11, 2006 and ended on July 31, 2006 (a total of 21 d). The N balance trial started immediately after and ended on August 6, 2006 (a total of 7 d).

Animals and Diets

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee (Protocol Nº F05-024/2). Pigs were cared for following the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

For this study, ninety-six [Yorkshire x Landrace] x Duroc piglets (48 barrows and 48 gilts) weaned at 17 ± 1 d were obtained from Genesus Genetics (Oakville, MB, Canada). After 5 d of adaptation to a commercial starter diet the average initial BW was 7.23 ± 0.19 kg. Pigs were weighed and blocked on the basis of BW and sex and randomly allotted into groups of four pigs per pen (2 barrows and 2 gilts). Each pen was considered the experimental unit. There were a total of 24 experimental units.

Six experimental units were assigned to one of the four dietary treatments; diets were a combination of two factors: 1) diet type: Corn-SBM diet (US), or a Corn-Wheat-Barley-Peas-SBM (WC); and 2) SID TLR (15%; low or 19%; high). Before mixing all the N-containing ingredients were sampled and sent to Degussa Canada (Burlington, ON, Canada) to be analyzed for CP and AA content (Table 3.1). Diets were formulated (Table 3.2) to differ only in TLR by adjusting the Trp level using L-Trp supplementation (Table 3.3). The other nutrients were supplied in amounts meeting or exceeding NRC (1998) recommendations using Rademacher et al. (2000) SID coefficients.

Table 3.1. Analyzed [a] CP and AA content of N-content ingredients (as-fed basis) of Experiment 1

	N – Content Ingredient							
Nutrient, g/kg	Wheat	Corn	Fish meal	SBM	Barley	Peas	Dried whey	SDBP
Dry Matter	873.1	857.9	905.4	883.3	856.6	870.2	972.9	891.3
Crude Protein	147.5	79.7	641.8	450.7	104.1	199.2	109.4	725.0
Methionine	2.3	1.6	18.2	6.3	1.7	1.8	1.8	6.3
Cystine	3.2	1.7	5.8	7.0	2.4	3.0	2.5	23.1
Methionine + Cystine	5.5	3.3	23.8	13.2	4.1	4.8	4.2	29.4
Lysine	4.0	2.5	49.9	28.2	3.6	15.8	9.1	66.4
Threonine	4.2	2.9	26.5	17.8	3.4	7.7	7.6	41.7
Tryptophan	1.7	0.6	7.6	6.2	1.3	1.9	1.9	13.3
Arginine	6.9	3.8	36.3	32.8	5.1	18.2	2.8	43.4
Isoleucine	4.9	2.7	26.7	20.2	3.5	8.9	6.8	27.1
Leucine	9.7	9.4	47.0	34.0	6.9	15.1	11.2	71.7
Valine	6.1	2.7	31.3	21.6	4.9	9.6	6.2	47.4
Histidine	3.4	2.3	18.9	12.2	2.3	5.1	2.1	27.5
Phenylalanine	6.6	2.9	26.0	22.7	5.1	9.8	4.1	41.9
Glycine						8.9	2.3	26.8
Serine						9.7	5.4	39.9
Proline						8.3	6.8	42.5
Alanine						9.2	5.5	41.1
Aspartic acid						24.4	12.1	72.0
Glutamic acid						34.2	19.6	103.2

[a] Wheat, corn, fish meal, SBM and barley were analyzed using NIR spectroscopy as described by Fontaine et al. (2002, 2004). Peas, dried whey and SDBP were analyzed using wet chemistry following the guidelines of Commission Directive (1998, 2000).

The experimental diets were mixed at the University of Manitoba feed mill. All the ingredients were obtained from local suppliers as per normal procedure. Wheat, pea and barley were coarsely ground (~800µm) prior to mixing the diets. To accomplish proper AA mixing, the crystalline AA (with the exception of Trp) were pre-mixed with 10 kg of corn (a portion of the diet formulation). The corn-AA premix was mixed with the rest of the ingredients. Each diet type was made up to 600 kg. Each diet type was split into 2 batches (300 kg each). From one batch of each diet type, 10 kg were taken and pre-mixed with crystalline L-Trp (L–

Table 3.2. Composition of experimental diets a (as-fed basis) used in Experiment 1.

Ingredient, %	US-Low	US-High	WC-Low	WC-High
Corn	59.88	59.83	25.00	25.00
Barley	----	----	10.00	10.00
SBM	14.39	14.39	4.46	4.46
Peas	----	----	5.00	5.00
Wheat	----	----	30.02	29.97
SDBP	4.00	4.00	4.00	4.00
Dried whey	8.00	8.00	8.00	8.00
Fish meal	6.00	6.00	6.00	6.00
Vegetable oil	4.23	4.23	3.63	3.63
Limestone	0.76	0.76	0.79	0.79
Dicalcium phosphate	0.58	0.58	0.58	0.58
Vitamin/mineral premix [b]	1.00	1.00	1.00	1.00
Biolys®	0.59	0.59	0.83	0.83
DL-Methionine	0.26	0.26	0.27	0.27
L-Threonine	0.18	0.18	0.24	0.24
L-Tryptophan	----	0.05	----	0.05
L-Isoleucine	0.13	0.13	0.18	0.18

[a] US-Low: Corn-SBM, Trp-to-Lys ratio 15%; US-High Corn-SBM, Trp-to-Lys ratio 19%; WC-Low: Corn-Wheat-Barley-Peas-SBM, Trp-to-Lys ratio 15%; WC-High: Corn-Wheat-Barley-Peas-SBM, Trp-to-Lys ratio 19%.

[b] Provided the following per kilogram of diet: vitamin A, 8255 IU; vitamin D_3, 1000 IU; vitamin E, 20 IU; vitamin K, 1.5 mg; riboflavin, 7.5 mg; niacin, 30 mg; vitamin B_{12}, 25 µg; pyridoxine, 4.5 µg; biotin, 200 µg; folic acid, 1 mg; thiamin, 4 mg; choline, 781 mg; copper, 10 mg; iodine, 0.6 mg; iron, 130 mg; manganese, 40 mg; selenium, 0.3 mg; zinc, 130 mg.

tryptophan, Evonik–Degussa, Hanau, Germany). Then the 10 kg aliquot with Trp was mixed with the rest of the diet (~290 kg) in a horizontal mixer for 10 min. In this way each diet type had the planed two levels of Trp supplementation (low and high).

Pigs were group housed in floor pens (length, 1.8 m; x width 1.2 m; x height, 0.9 m height) with plastic-covered expended metal sheet flooring in a temperature controlled room (starting at 30ºC in wk one; temperature was reduced 1ºC/wk) with

Table 3.3. Nutrient content of experimental diets a (as-fed basis) of Experiment 1.

Nutrient	US-Low	US-High	WC-Low	WC-High
Dry Matter, g/kg [b]	903.9	896.9	904.6	898.5
Crude fat, g/kg [c]	71.16	71.16	58.48	58.48
CP, g/kg [b]	208.2	204.3	199.8	198.7
Crude fiber, g/kg [c]	23.14	23.14	24.59	24.59
Metabolizable Energy, MJ/kg [c]	14.57	14.57	14.48	14.48
Net Energy, MJ/kg [c]	10.7	10.7	10.7	10.7
Amino acids, g/kg [b]				
Methionine	6.3	6.2	6.3	6.2
Cystine	3.5	3.4	3.5	3.5
Methionine + Cystine	9.8	9.6	9.8	9.7
Lysine	14.9	14.8	14.6	15.0
Threonine	10.6	10.4	10.1	10.1
Tryptophan	2.5	2.9	2.4	2.8
Arginine	11.4	11.1	10.1	10.2
Isoleucine	9.3	9.2	9.3	9.1
Leucine	17.6	17.4	15.2	15.0
Valine	10.1	10.0	9.6	9.5
Histidine	5.2	5.2	4.7	4.7
Phenylalanine	9.4	9.3	8.9	8.9
Glycine	8.8	8.6	8.6	8.5
Serine	9.7	9.4	8.5	8.6
Proline	11.9	11.5	13.3	12.9
Alanine	11.4	11.2	9.9	9.8
Aspartic acid	19.3	18.9	15.8	15.6
Glutamic acid	32.6	31.5	36.0	35.9
Trp-to-Lys ratio, % (total AA basis) [b]	16.78	19.59	16.44	18.67
Trp-to-Lys ratio, %(SID basis) [c]	15.96	19.09	15.53	18.36

[a] US-Low: Corn-SBM, Trp-to-Lys ratio 15%; US-High Corn-SBM, Trp-to-Lys ratio 19%; WC-Low: Corn-Wheat-Barley-Peas-SBM, Trp-to-Lys ratio 15%; WC-High: Corn-Wheat-Barley-Peas-SBM, Trp-to-Lys ratio 19%.
[b] Diets were analyzed using wet chemistry following the guidelines of Commission Directive (1998, 2000)..
[c] Calculated values.

a 12 h light/dark cycle. Pigs were allowed *ad libitum* access to feed and water through a nipple drinker and a feeder. Diets were offered as mash. The performance trial was conducted over a 3-wk period. Feed disappearance and pig

live weight were monitored weekly and the two parameters used to determine ADG, ADFI and GFR for each experimental unit.

Every wk, blood samples were obtained from one pig per pen for the determination of PUN.

Immediately after the performance trial, three barrows per treatment were selected (according to their BW, in such a way that they were close to the treatment average) for a N balance trial. Pigs were housed in stainless-steel adjustable metabolic crates (length, 1.8 m; x width, 0.6 m; x height, 0.8 m). These crates allow separate collection of urine and feces. Following the recommendations of Adeola (2001), pigs were fed constantly at 2.6 times their maintenance energy requirement. Daily feed allowance was divided into two equal meals (0830 and 1530). After a 3 d adaptation period, total feces and urine were collected during 4 d (i.e. on the 4^{th}, 5^{th}, 6^{th}, and 7^{th} d). On d 4 (first day of feces collection) and d 8 (one day after the feces collection period) ferric oxide was added to the diets (1 g of ferric oxide was mixed with 100 g of diet). This mixture was fed to the pig at 0830. The rest of its feed allowance was provided, once the pig finished the mixture of ferric oxide and diet (Adeola, 2001) as a marker to determine the start and end of the feces collection period. The first appearance of red feces marked the start of the collection period. This period lasted until the reappearance of red feces. Urine collection started on the fourth day and ended on the seventh day. Diets, feces and urine samples were analyzed for N content to determine N ingestion, N excretion and N retention.

Sample preparation and chemical analyses

Samples (~300 g) of N-containing ingredients (i.e. wheat, corn, fish meal, SBM, barley, peas, sprayed dried blood plasma (SDBP) and dried whey) and diets where sent to Degussa Canada (Burlington, ON, Canada) for CP and AA analysis. Wheat, corn, fish meal, SBM and barley were analysed using near-infrared (NIR) spectroscopy, following the procedures described by Fontaine et al. (2002, 2004; Table 3.1). The AA analysis for peas, SDBP, dried whey and diets were performed as described by Nyachoti et al. (2006) and following the guidelines of Commission Directive (1998, 2000).

For PUN samples, 10 ml of blood was collected from one pig per pen via jugular vein puncture into sodium-heparin-vacuntainer-tubes (Becton Dickinson, Rutherford, NJ) on days 0, 7, 14 and 21. Immediately after collection, the vacuntainer tubes were kept on ice until process. Once all the pigs were bled, the tubes were centrifuged at 1500 rpm for 10 min at 0ºC to recover plasma (Shriver et al., 2003; Nyachoti et. al., 2006). After centrifugation, 2 ml of plasma were taken using disposable Pasteur pipettes and placed into 2 ml cryogenic vial (Fisher Scientific, Ottawa, ON). Vials were kept frozen (-20ºC) until analysis. PUN was determined by Nova Stat profile M blood gas and electrolyte analyzer (Nova Biomedical Corporation, Waltham, MA). PUN concentrations are obtained by measuring the acidity and gas tensions in blood plasma.

Urine was collected into plastic jugs containing 20 ml of 3 N *HCl* to reduce the pH and avoid the loss of N as ammonia (Adeola, 2001). In the morning of collection days, urine was weighed, recorded, and a subsample (30% aliquot) was

taken. Subsamples were filtered through glass wool to eliminate particulate matter (Adeola, 2001) and kept frozen (-20ºC). The four sub samples (i.e. one for each day of collection) were thawed and pooled together. A new subsample was taken (30% aliquot) and kept frozen until analysis.

Feces were collected starting from the first appearance of red feces and before the reappearance of red feces. Feces were collected, weighed, sealed in plastic bags, and kept frozen (-20ºC). At the end of collection, feces were thawed and a subsample (20%) was taken. Subsamples were placed in a forced-draft oven at 55ºC for at least 5 d. Dried fecal samples were ground to pass through a 1-mm screen using a Willey mill (Arthur H. Thomas, Philadelphia, PA) and stored in sealed plastic bags until analysis (Adeola, 2001).

At the beginning of the N balance study, a 300 g of each diet was taken. Samples were ground in a Willey mill (Arthur H. Thomas, Philadelphia, PA) using a 1-mm screen. Ground diet samples, feces and urine samples were analyzed for N content using a LECO CNS-2000 Elemental Analyzer (LECO Corp., St. Joseph, MI).

Calculations

In order to calculate N ingestion, N retention and N excretion, feed intake was averaged to grams per day (as-fed basis). The amount of N in urine and feces was also averaged to grams per day (as-fed basis).

N ingestion (g/d, as-fed basis) was calculated using the following equation:

$$NI = (FI \times N_{(diet)})/ 100$$

where: NI= N ingestion (g/d, as-fed basis); FI = feed intake (g/d, as-fed basis); and $N_{(diet)}$ = amount of N in the diet (%, as-fed basis).

N excretion (g/d, as-fed basis) was calculated using the following equation:

$$NE=N_{(urine)}+ N_{(feces)}$$

where: NE= N excretion (g/d, as-fed basis); $N_{(urine)}$= amount of N in urine (g/d, as-fed basis); and $N_{(feces)}$= amount of N in feces (g/d, as-fed basis)

N retention (g/d, as-fed basis) was calculated using the following equation:

$$NR = NI – NE$$

where: NR= N retention (g/d, as-fed basis); NI = N ingestion (g/d, as-fed basis); and NE= N excretion (g/d, as-fed basis).

N excretion (%, as-fed basis) was calculated using the following equation:

$$NE\% = (NE / NI) \times 100$$

where: NE%= N excretion (%, as-fed basis); NE= N excretion (g/d, as-fed basis); and NI= N ingestion (g/d, as-fed basis).

N retention (%, as-fed basis) was calculated using the following equation:

$$NR\% = 100 – NE\%$$

where: NR%= N retention (%, as-fed basis); and NE%= N excretion (%, as-fed basis).

3.3.2 Experiment 2

Timeline

Pigs arrived to the TKCCAR in 2 batches. The fist batch arrived on February 12, 2007. After 2 d of adaptation, the performance trial started on February 14,

2007 and ended on March 6, 2007 (a total of 21 d). The N balance trial started immediately after and ended on March 13, 2007 (a total of 7 d). The second batch arrived on March 26, 2007. After 2 d of adaptation, the performance trial for the second batch started on March 28, 2007 and ended on April 17, 2007 (21 d); the N balance started the same day and ended on April 25, 2007 (7 d).

Animals and Diets

Experimental procedures and care of the animals were the same as per Experiment 1.

For this study, ninety-six Cotswold piglets (48 barrows and 48 gilts) weaned at 17 ± 1 d were obtained from the University of Manitoba's Glenlea Swine Research Farm. Pigs arrived to the TKCCAR in two batches (each batch consisted of 48 pigs; half of them were barrows and half gilts).

For each batch, after 2 d of adaptation to a commercial starter diet, 2 spare pigs (1 barrow and 1 gilt) were removed based on their BW (the heaviest and lightest from each gender). The average initial BW was 6.90 ± 0.28 kg. Pig allotment was similar to Experiment 1.

Within each batch, three experimental units were assigned to one of the four dietary treatments. Diets were a combination of two factors: 1) SID TLR (16% or 20%) and 2) CP level (low; 17% or high; 20%). Sampling of diets and N-containing ingredients (i.e. corn, fish meal, SBM, dried whey and SDBP; Table 3.4) were done as described for Experiment 1. Diets, a corn-SBM base, were formulated (Table 3.5) to differ only in their TLR and the CP level. All other nutrients were supplied in amounts meeting or exceeding NRC (1998)

Table 3.4. Analyzed[a] CP and AA contents of N-containing ingredients (as-fed basis) of Experiment 2.

	N – Containing Ingredient				
Nutrient, g/kg	Corn	Fish meal	SBM	Dried whey	SDBP
Dry Matter	877.1	926.3	903.1	954.6	876.3
Crude Protein	82.2	674.4	455.1	118.0	722.5
Methionine	1.6	17.3	5.8	1.6	5.5
Cystine	1.9	5.0	6.4	2.4	22.1
Methionine + Cystine	3.5	22.4	12.2	3.9	27.5
Lysine	2.6	49.1	2.75	8.8	61.4
Threonine	2.9	26.2	17.3	7.2	38.8
Tryptophan [b]	0.6	7.6	6.2	1.9	12.7
Arginine	3.8	37.5	32.6	2.4	41.2
Isoleucine	2.7	26.0	19.7	6.4	24.6
Leucine	9.3	46.1	33.6	10.4	67.6
Valine	3.8	30.3	20.8	6.0	45.4
Histidine	2.3	18.3	12.6	2.2	24.2
Phenylalanine	3.9	25.6	21.9	3.5	37.9
Glycine	3.2	43.2	18.6	2.2	25.2
Serine	3.8	24.5	22.2	5.2	39.5
Proline	6.9	28.9	22.9	6.9	41.8
Alanine	5.9	40.8	19.2	5.1	37.8
Aspartic acid	5.4	57.4	49.5	11.1	66.2
Glutamic acid	14.0	81.8	78.2	18.6	99.6
Ammonia	1.9	10.4	8.5	2.8	9.7

[a] Ingredients were analyzed using wet chemistry following the guidelines of Commission Directive (1998, 2000).
[b] Calculated values.

recommendations using Rademacher et al. (2000) SID coefficients (Table 3.6).

The experimental diets were mixed at the University of Manitoba feed mill. All the ingredients were obtained from local suppliers. To accomplish proper AA mixing, crystalline AA, including crystalline L-Trp (L–tryptophan, Evonik–

Table 3.5. Composition of experimental diets [a] (as-fed basis) used in Experiment 2.

Ingredient, %	H-16	H-20	L-16	L-20
Corn	59.07	59.04	67.12	67.08
SBM	15.10	15.09	6.00	6.00
SDBP	4.00	4.00	4.00	4.00
Dried whey	8.00	8.00	8.00	8.00
Fish meal	6.00	6.00	6.00	6.00
Vegetable oil	4.23	4.23	4.23	4.23
Limestone	0.68	0.68	0.64	0.64
Dicalcium phosphate	0.58	0.58	0.71	0.71
Vitamin/mineral premix [b]	1.00	1.00	1.00	1.00
Biolys®	0.59	0.59	1.02	1.02
DL-Methionine	0.37	0.37	0.45	0.45
L-Threonine	0.13	0.13	0.25	0.25
L-Tryptophan	0.01	0.07	0.06	0.11
L-Isoleucine	0.13	0.13	0.27	0.27
L-Valine	0.11	0.11	0.26	0.25

[a] **H-16**: Trp-to-Lys ratio =16%, High CP = 20%; **H-20**: Trp-to-Lys ratio = 20%, High CP = 20%; **L-16**: TRP-to-LYS ratio = 16%, Low CP = 17%; **L-20**: TRP-to-LYS ratio = 20%, Low CP = 17%

[b] Contributed the following per kilogram of diet: vitamin A, 8255 IU; vitamin D_3, 1000 IU; vitamin E, 20 IU; vitamin K, 1.5 mg; riboflavin, 7.5 mg; niacin, 30 mg; vitamin B_{12}, 25 µg; pyridoxine, 4.5 µg; biotin, 200 µg; folic acid, 1 mg; thiamin, 4 mg; choline, 781 mg; copper, 10 mg; iodine, 0.6 mg; iron, 130 mg; manganese, 40 mg; selenium, 0.3 mg; zinc, 130 mg.

Degussa, Hanau, Germany), were initially pre-mixed with ~10 kg of ground corn (a portion of the diet formulation). The corn-AA premix was included on the feed mill mixing recipe. After mixing all the ingredients, each diet was then pelleted at a temperature of 70ºC and pressure load of 121 lbs for 40 min.

Pigs were housed as described for Experiment 1. Diets were offered in a pelleted form. ADG, ADFI and GFR were calculated as described for Experiment 1. Blood samples were obtained weekly from one pig per pen to determine PUN.

Within each batch, after the performance trial, two barrows per treatment

Table 3.6. Nutrient content of experimental diets [a] (as-fed basis) of Experiment 2.

Nutrient	H-16	H-20	L-16	L-20
Dry Matter, g/kg [b]	897.1	897.4	893.0	918.7
Crude fat, g/kg [c]	74.3	74.3	76.1	76.1
CP, g/kg [b]	209.9	211.0	190.8	189.8
Crude fiber, g/kg [c]	27.2	27.2	20.4	20.4
Metabolizable Energy, MJ/kg [c]	14.7	14.7	14.6	14.6
Net Energy, MJ/kg [c]	10.9	10.9	11.1	11.1
Amino acids, g/kg [b]				
Methionine	8.3	7.9	8.4	8.5
Cystine	3.7	3.7	3.3	3.3
Methionine + Cystine	12.1	11.6	11.8	11.8
Lysine	14.0	14.6	14.2	13.7
Threonine	11.0	10.8	11.0	11.0
Tryptophan	2.6	3.1	2.7	3.3
Arginine	10.9	11.0	8.9	8.9
Isoleucine	9.1	8.9	9.2	9.6
Leucine	17.3	17.5	15.6	15.7
Valine	10.5	10.4	10.7	11.2
Histidine	5.1	5.2	4.5	4.5
Phenylalanine	9.1	9.3	8.0	8.0
Glycine	8.9	8.9	7.7	7.7
Serine	10.0	10.1	8.6	8.5
Proline	11.8	11.5	10.6	10.3
Alanine	11.3	11.4	10.4	10.3
Aspartic acid	18.5	18.7	15.3	15.2
Glutamic acid	31.9	32.2	27.1	26.8
Ammonia	3.9	4.0	3.4	3.8
Trp-to-Lys ratio, % (total AA basis) [b]	18.6	21.2	19.0	24.1
Trp-to-Lys ratio, %(SID basis) [b]	17.6	20.7	18.4	23.9

[a] **H-16**: Trp-to-Lys ratio =16%, High CP = 20%; **H-20**: Trp-to-Lys ratio = 20%, High CP = 20%; **L-16**: TRP-to-LYS ratio = 16%, Low CP = 17%; **L-20**: TRP-to-LYS ratio = 20%, Low CP = 17%.

[b] Diets were analyzed using wet chemistry following the guidelines of Commission Directive (1998, 2000)..

[c] Calculated values.

were selected (according to their BW, in such a way that they were close to the treatment average) for the N balance trial. Pigs were housed as described for Experiment 1. Feed allowance, urine, and feces collection were done as described in Experiment 1.

Diets, feces and urine samples were analyzed for nitrogen content to determine: N ingestion, N excretion and N retention.

Sample preparation and chemical analyses

Samples (~300 g) of N-containing ingredients (i.e. corn, fish meal, SBM, SDBP and dried whey) and diets where sent to Degussa Canada (Burlington, ON, Canada) for CP and AA analysis as described by Nyachoti et al. (2006). PUN (for the performance trial), urine and feces (for the N balance) samples were obtained and processed as described in Experiment 1.

Calculations

Calculations were done as described in Experiment 1.

3.3.3 Statistical Analysis

Before analyzing the data, ADFI and ADG were calculated using the Proc REG of SAS (SAS Inst., Inc., Cary, NC) as the slope of the regression line for feed intake or BW, respectively, over time. GFR was determined as the quotient of the division of ADG and ADFI, where ADFI was the divisor. Averaged PUN was calculated as the average of the PUN measurements done in wk 1, 2 and 3.

Residuals of data were analyzed using the Proc Univariate of SAS (SAS Inst., Inc., Cary, NC) to confirm they met the assumptions of ANOVA. Normality was tested using the Shapiro-Wilk test.

In Experiment 1, data was analyzed as a completely randomized design (CRD) with a 2 x 2 factorial arrangement of treatments using Proc GLM of SAS (SAS Inst., Inc., Cary, NC). The model used was $y_{ijk} = \mu + d_i + r_j + dr_{ij} + e_{ijk}$.

Where y_{ijk} = initial BW, final BW, ADG, ADFI, GFR, initial PUN, averaged PUN, Final PUN, N ingestion, N excretion or N retention of the k'th pen of the i'th diet type and the j'th SID TLR; μ = population mean; d_i = main effect of the i'th diet type; r_j = main effect of the j'th SID TLR; dr_{ij} = interaction effects of the i'th diet type and the j'th SID TLR; and e_{ijk} = residual error of the k'th pen of the i'th diet type and the j'th SID TLR. In Experiment 2, data was analyzed as a completely randomized block design (RBD) with a 2 x 2 factorial arrangement of treatments using Proc GLM of SAS (SAS Inst., Inc., Cary, NC). Weanling arrival batch was considered the blocking factor. The model used was $y_{ijk(l)} = \mu + r_i + p_j + rp_{ij} + b_l + e_{ijk(l)}$. Where $y_{ijk(l)}$ = initial BW, final BW, ADG, ADFI, GFR, initial PUN, averaged PUN, Final PUN, N ingestion, N excretion and N retention of the k'th pen of the i'th SID TLR and the j'th protein level within the l'th batch; μ = population mean; r_i = main effect of the i'th SID TLR; p_j = main effect of the j'th CP level; rp_{ij} = interaction effects of the i'th SID TLR and the j'th CP level; b_l = effect of the l'th batch; and $e_{ijk(l)}$ = residual error of k'th pen of the i'th SID TLR and the j'th protein level within the l'th batch. In both experiments, for the averaged PUN analysis, the model also included initial PUN as a covariance, when a significant F-value ($P < 0.10$) was indicated by the ANOVA, the Scheffe test was used to compare treatments means.

3.4 RESULTS

3.4.1 Experiment 1

Nutritional content of experimental diets was in close agreement with the proposed values. The proposed total Lys was 1.50%, the analysed level was 1.48 ± 0.02% (on average). Total proposed Trp levels were 0.24% and 0.29% for the lower and higher TLR, respectively. Analysed values were 0.25 ± 0.01% and 0.29 ± 0.01% on average for lower and higher TLR, respectively (Table 3.3). All animals remained healthy during the experimental period.

There were no differences ($P > 0.10$) on the initial BW (7.30 ± 0.05 kg), ADFI or (538.50 ± 7.26 g/d) due to diet type or Trp level of inclusion. The US diet had a higher ($P = 0.04$) final BW (15.36 kg) than the WC diet (17.80 kg). Similarly, pigs fed the US diet gained more weight ($P = 0.01$) than those fed the WC diet (0.39 vs. 0.36 kg/d). The pigs fed the US diet had a better ($P = 0.06$) GFR than those fed the WC diet (0.712 vs. 0.671 kg BW / kg feed). No differences were found ($P > 0.10$) between the two levels of Trp on final BW (15.08 ± 0.23 kg), or ADG (0.37 ± 0.01 kg/d). There were no effects of the interaction between diet type and TLR on initial BW, final BW, ADFI, ADG or GFR (Table 3.7).

No differences ($P > 0.10$) were found on the initial PUN (4.49 ± 0.83 mmol/l) or final PUN (1.808 ± 0.37 mmol/l) due to diet type. No differences ($P > 0.10$) were found on the initial PUN or average PUN (1.93 ± 0.34 mmol/l) due to TLR. Pigs fed the high TLR diets had higher ($P = 0.06$) final PUN (1.94 ± 0.45 mmol/l) than those fed the low TLR (1.68 ± 0.19 mmol/l). Similarly, pigs fed the US diet type had lower ($P = 0.07$) average PUN (1.79 ± 0.24 mmol/l) than those fed the WC

Table 3.7. Performance of early-weaned pigs fed two types of diets and two levels of Trp-to-Lys ratio.

	Diet Type [v]		TLR [w]		SEM	Interactions [x]				SEM	*P*-Values [y]		
						US		WC					
	US	WC	Low	High		Low	High	Low	High		DT [v]	TLR	DT x TLR
Initial BW, kg	7.28	7.33	7.36	7.24	0.054	7.36	7.19	7.36	7.30	0.076	0.519	0.131	0.499
Final BW, kg	15.36 [a]	14.80 [b]	15.24	14.92	0.185	15.49	15.23	14.98	14.91	0.261	0.043	0.238	0.843
ADFI, g/d	547	530	540	537	9.717	545	550	535	525	13.742	0.213	0.877	0.587
ADG, kg/d	0.389 [a]	0.355 [b]	0.376	0.368	0.009	0.390	0.387	0.362	0.349	0.012	0.012	0.518	0.662
GFR	0.712 [a]	0.671 [b]	0.698	0.684	0.015	0.718	0.706	0.678	0.663	0.021	0.060	0.514	0.966
N [z]	12	12	12	12		6	6	6	6				

[a,b] Means with different superscript are statistically different ($P < 0.10$)

[v] Diet type: US: Corn-SBM; WC: Corn-wheat-barley-peas-SBM

[w] Trp-to-Lys ratio (SID basis): Low = 15%; High = 19%

[x] LSMeans

[y] Main effects of diet type (DT), Trp-to-Lys ratio (TLR) or the interaction (DT x TLR)

[z] Each replicate represents the average of 4 pigs (2 barrows and 2 gilts).

diet type (2.06 ± 0.38 mmol/l). There were no effects ($P > 0.10$) of the interaction of both factors over the PUN measurements. No differences ($P > 0.05$) were found due to diet type or TLR on N ingestion (23.02 ± 0.47 g/d) and N excretion (4.57 ± 0.17 g/d; or 20.09 ± 1.10 %) Pigs fed the low TLR diets retained more ($P = 0.09$) N (18.86 ± 1.39 g/d) than those fed the high TLR diets (17.52 ± 0.98 g/d). This difference was not observed ($P > 0.10$) when the data was expressed as a percentage of the N intake (79.91 ± 1.10%, on average for the high and low TLR diets). No effects ($P > 0.10$) of the interaction between diet type and TLR were found for any of the parameters measured on the N balance study (Table 3.8).

3.4.2 Experiment 2

All of the animals remained healthy during the experiment, with the exception of one gilt from batch 1 that died during blood sampling at the beginning of the second wk, There were no effects ($P > 0.10$) of arrival batch in ADFI, ADG, GFR, final PUN, averaged PUN and N ingestion (data not shown). Batch 2 had higher ($P < 0.01$) initial BW (7.17 ± 0.07 kg), final BW (16.77 ± 0.80 kg) and N retention (17.22 ± 0.93 g/d or 79.02 ± 2.41 %) than batch 1 (6.63 ± 0.07 kg, 15.52 ± 1.22 kg, and 15.02 ± 1.38 g/d or 69.28 ± 7.82 %, respectively; data not shown). Batch 1 that had higher ($P < 0.01$) initial PUN (4.36 ± 1.35 mmol/l) and N excretion (6.73 ± 1.94 g/d or 30.72 ± 7.82 %) than batch 2 (2.60 ± 0.70 mmol/l, and 4.60 ± 0.80 g/d or 20.98 ± 2.41 %, respectively; data not shown).

There were no differences ($P > 0.10$) due to the diverse TLR or CP levels of the diets on initial BW (6.90 ± 0.02 kg), final BW (16.15 ± 0.25 kg) or ADFI (519.75 ± 3.03 g/d). The high-CP diet showed better ($P = 0.001$) ADG (0.47 vs.

Table 3.8. Plasma urea N concentration and N balance in early-weaned pigs fed two types of diets and two levels of Trp-to-Lys ratio.

	Diet Type [u]		TLR [v]		SEM	Interactions [w]				SEM	*P*-Values [x]		
						US		WC					
	US	WC	Low	High		Low	High	Low	High		DT [u]	TLR	DT x TLR
PUN, mmol/l													
Initial	4.408	4.575	4.617	4.367	0.245	4.333	4.483	4.900	4.250	0.347	0.636	0.480	0.263
Final	1.700	1.917	1.675 [a]	1.942 [b]	0.096	1.650	1.750	1.700	2.130	0.136	0.127	0.064	0.235
Average	1.794 [a]	2.054 [b]	1.972	1.877	0.097	1.839	1.743	2.113	2.001	0.139	0.073	0.497	0.962
n [y]	12	12	12	12		6	6	6	6				
N Balance [z]													
Ing, g/d	23.21	22.32	23.23	23.30	0.579	23.63	22.79	22.83	21.81	0.819	0.307	0.289	0.914
Exc, g/d	4.58	4.57	4.37	4.78	0.367	4.52	4.64	4.22	4.92	0.519	0.985	0.449	0.593
Exc, %	19.70	20.49	18.80	21.39	1.407	19.03	20.36	18.56	22.41	1.989	0.703	0.230	0.544
Ret, g/d	18.63	17.75	18.86 [a]	17.52 [b]	0.492	19.12	18.15	18.61	16.89	0.696	0.240	0.090	0.599
Ret, %	80.30	79.52	81.20	78.62	1.407	80.97	79.64	81.44	77.59	1.989	0.703	0.230	0.544
n [y]	6	6	6	6		3	3	3	3				

[a,b] Means with different superscript are statistically different ($P < 0.10$)

[u] Diet type: US: Corn-SBM; WC: Corn-wheat-barley-peas-SBM.

[v] Trp-to-Lys ratio (SID basis): Low = 15%; High = 19%.

[w] LSMeans.

[x] Main effects of diet type (DT), Trp-to-Lys ratio (TLR) or the interaction (DT x TLR).

[y] Each replicate represents one pig.

[z] Ing = ingestion; Exc = excretion; Ret = retention; each replicate represents one barrow.

0.42 kg/d) and better GFR (0.90 vs. 0.81 kg BW/kg feed) than the low-CP diet. No differences ($P > 0.10$) due to the TLR were observed on ADG (0.44 ± 0.01 kg/d) or GFR (0.85 ± 0.01 kg BW/kg feed). No effects ($P > 0.10$) of the interactions of both factors were found on the performance parameters evaluated (Table 3.9).

All of the animals remained healthy during the experiment, with the exception of one gilt from batch 1 that died during blood sampling at the beginning of the second wk, there were no effects ($P > 0.10$) of weanling arrival batch on ADFI, ADG, GFR, final PUN, averaged PUN and N ingestion (data not shown). Batch 2 had higher ($P < 0.01$) initial BW (7.17 ± 0.07 kg), final BW (16.77 ± 0.80 kg) and N retention (17.22 ± 0.93 g/d or 79.02 ± 2.41 %) than batch 1 (6.63 ± 0.07 kg, 15.52 ± 1.22 kg, and 15.02 ± 1.38 g/d or 69.28 ± 7.82 %, respectively; data not shown). Batch 1 that had higher ($P < 0.01$) initial PUN (4.36 ± 1.35 mmol/l) and N excretion (6.73 ± 1.94 g/d or 30.72 ± 7.82 %) than batch 2 (2.60 ± 0.70 mmol/l, and 4.60 ± 0.80 g/d or 20.98 ± 2.41 %, respectively; data not shown).

There were no differences ($P > 0.10$) due to the diverse TLR or CP levels of the diets on initial BW (6.90 ± 0.02 kg), final BW (16.15 ± 0.25 kg) or ADFI (519.75 ± 3.03 g/d). The high-CP diet showed better ($P < 0.01$) ADG (0.47 vs. 0.42 kg/d) and better GFR (0.90 vs. 0.81 kg BW/kg feed) than the low-CP diet. No differences ($P > 0.10$) due to the TLR were observed on ADG (0.44 ± 0.01 kg/d) or GFR (0.85 ± 0.01 kg BW/kg feed). No effects ($P > 0.10$) of the interactions of both factors were found on the performance parameters evaluated (Table 3.9).

No differences ($P > 0.10$) were found in the initial PUN (3.48 ± 0.03 mmol/l) due to CP level or TLR. The low-CP diet showed lower ($P < 0.05$) final PUN (1.34

Table 3.9. Performance of early-weaned piglets fed two levels of CP and two levels of Trp-to-Lys ratio.

	CP [v]		TLR [w]		SEM	Interactions [x]				SEM	*P*-Values [y]		
						Low-CP		High-CP					
	Low	High	16%	20%		TLR 16%	TLR 20%	TLR 16%	TLR 20%		CP	TLR	CP x TLR
Initial BW, kg	6.89	6.92	6.88	6.92	0.019	6.86	6.91	6.89	6.94	0.027	0.291	0.100	0.964
Final BW, kg	15.85	16.45	16.19	16.11	0.305	15.88	15.82	16.50	16.39	0.431	0.182	0.848	0.945
ADFI, g/d	518	522	517	523	7.801	522	514	511	532	11.03	0.728	0.573	0.202
ADG, g/d	0.420 [b]	0.467 [a]	0.439	0.448	0.007	0.424	0.417	0.454	0.479	0.011	0.001	0.441	0.156
GFR	0.812 [b]	0.895 [a]	0.850	0.857	0.011	0.813	0.811	0.888	0.902	0.015	< 0.001	0.682	0.613
N [z]	12	12	12	12		6	6	6	6				

[a,b] Means with different superscript are statistically different ($P < 0.10$).

[v] Crude protein level; Low = 17%, High = 20%.

[w] Trp-to-Lys ratio.

[x] LSMeans.

[y] Main effects of CP level (CP), Trp-to-Lys ratio (TLR) or the interaction (CP x TLR). There were no effects ($P > 0.10$) of arrival batch in all the performance parameters evaluated; except for batch 2 that had higher ($P < 0.01$) initial BW (7.17 $\pm$ 0.07 kg) and final BW (16.77 $\pm$ 0.80 kg) than batch 1 (6.63 $\pm$ 0.07 kg and 15.52 $\pm$ 1.22, respectively).

[z] Each replicate represents the average of 4 pigs (2 barrows and 2 gilts).

vs. 1.78 mmol/l) and averaged PUN (1.36 vs. 1.74 mmol/l) than the high-CP diet. There were no differences due to the TLR in the final PUN (1.56 ± 0.04 mmol/l) or averaged PUN (1.55 ± 0.02 mmol/l). No interaction effects ($P > 0.10$) of CP level and TLR were observed on initial, final or average PUN (Table 3.10).

No differences ($P > 0.10$) were found in N excretion (25.85 ± 2.10 %) or N retention (16.12 ± 0.33 g/d or 74.15 ± 2.10 %) due to CP level or TLR. The high-CP diet had higher ($P < 0.10$) N ingestion (22.82 vs. 20.76 g/d) and N excretion (6.41 vs. 4.93 g/d) than the low-CP diet; however, this difference on N excretion was not observed ($P > 0.10$) when the data was expressed as a percentage of the N intake (25.85 ± 1.94%, on average for the high and low TLR diets). No statistical differences ($P > 0.10$) were observed for N ingestion (21.79 ± 0.29 g/d), N excretion (5.67 ± 0.44 g/d; or 25.85 ± 1.94 %) or N retention (16.12 ± 0.41 g/d; or 74.15 ± 1.94 %) due to TLR. No interaction effects were found ($P > 0.05$) for all the parameters evaluated in the N balance study (Table 3.10).

3.5 DISCUSSION

3.5.1 Experiment 1

The purpose of the first experiment was to evaluate the effect of diet composition on the TLR. Two diets were formulated: a corn-SBM diet (USA type of diet; US) and a Corn-Wheat-Barley-Peas-SBM (Western Canada type of diet; WC). Each diet had two levels of SID TLR: a low one (15%) and a high one (19%). The close agreement between the calculated and analysed values confirmed

Table 3.10 Plasma urea N and N balance in early-weaned piglets fed two levels of CP and two levels of Trp-to-Lys ratio.

	CP [v]		TLR [w]		SEM	Interactions [x]				SEM	*P*-Values [y]		
						Low-CP		High-CP					
	Low	High	16%	20%		TLR 16%	TLR 20%	TLR 16%	TLR 20%		CP	TLR	CP x TLR
PUN, mmol/l													
Initial	3.508	3.450	3.508	3.450	0.326	3.767	3.250	3.250	3.650	0.461	0.901	0.901	0.333
Final	1.342 [a]	1.775 [b]	1.583	1.533	0.072	1.383	1.300	1.783	1.767	0.102	< 0.001	0.629	0.747
Average	1.361 [a]	1.742 [b]	1.537	1.566	0.064	1.375	1.344	1.696	1.789	0.090	0.001	0.750	0.421
n [y]	12	12	12	12		6	6	6	6				
N Balance [z]													
Ing, g/d	20.76 [b]	22.82 [a]	21.81	21.77	0.291	21.11	20.40	22.50	23.13	0.411	< 0.001	0.922	0.131
Exc, g/d	4.93 [b]	6.41 [a]	5.98	5.36	0.460	5.58	4.27	6.37	6.44	0.651	0.044	0.361	0.314
Exc, %	23.68	28.02	27.22	24.48	1.945	26.43	20.93	28.01	28.03	2.751	0.143	0.341	0.337
Ret, g/d	15.83	16.41	15.83	16.41	0.435	15.53	16.13	16.13	16.69	0.615	0.367	0.366	0.981
Ret, %	76.32	71.98	72.78	75.52	1.945	73.57	79.07	71.99	71.97	2.751	0.143	0.341	0.347
n	8	8	8	8		4	4	4	4				

[a,b] Means with different superscript are statistically different ($P < 0.10$).

[v] Crude protein level; Low = 17%, High = 20%.

[w] Trp-to-Lys ratio.

[x] LSMeans.

[y] Main effects of diet type (DT), Trp-to-Lys ratio (TLR) or the interaction (DT x TLR). There were no effects ($P > 0.10$) of arrival batch in all the parameters evaluated; except for batch 1 that had higher ($P < 0.01$) initial PUN (4.36 ± 1.35 mmol/l) and N excretion (6.73 ± 1.94 g/d or 30.72 ± 7.82 %) than batch 2 (2.60 ± 0.70 mmol/l, and 4.60 ± 0.80 g/d or 20.98 ± 2.41 %, respectively). Batch 2 had higher ($P < 0.10$) N retention (17.22 ± 0.93 g/d or 79.02 ± 2.41 %) than batch 1 (15.02 ± 1.38 g/d or 69.28 ± 7.82 %).

[z] Ing = ingestion; Exc = excretion; Ret = retention; each replicate (n) represents one barrow.

that diet mixing was effective and the chemical analysed were accurate for both diets.

Since the initial BW was not different among treatments there was no need to perform an analysis of covariance. In addition, all the variables of the study (i.e. ADG, ADFI, GFR, PUN, N ingestion, N excretion and N retention) were checked to ensure they met the assumptions of the analysis of variance (Steel et al., 1997).

Only barrows were selected for the N balance study because, anatomically, it is easier to collect urine and feces separately (Adeola, 2001). Barrows were selected based on BW proximity to the dietary treatment average, so they can be representative of their treatment.

ADFI was similar to previous studies (530 g/d Burgoon et al., 1992; 553 g/d, Han et al., 1993; 558.54 ± 54 g/d, Eder et al., 2001) and was also in close agreement with the NRC (1998) reported values for feed intake. As shown in Table 3.7 there was no effect of Trp supplementation on ADFI on both diet types. This was a surprise because previous research showed that Trp supplementation can increase feed intake due to a rise in serotonin synthesis (Henry et al., 1992, 1996; Eder et al., 2001). A recent study (Jansman et al., 2010) found a large response on feed intake towards Trp supplementation. The authors suggested that the increase in feed intake was due to an increase in serotonin concentration and an increase in ghrelin production. Serotonin synthesis is highly dependant on dietary Trp supplementation (Le Floc'h and Sève, 2007) and plays a key role in appetite regulation. On the other hand Trp increases the level of ghrelin in the GIT membrane (Zhang et al., 2007). Ghrelin is a hormone that can increase appetite (Kojima et al., 1999).

Although nutritional composition of both diet types was similar (Table 3.3) the pigs fed the WC diet had lower ($P < 0.10$) final BW, ADG, and GFR than those fed

the US diet. This can be attributed to the presence of ANF in the WC diet. Even though the level of ANF was not quantified in this experiment, it has been reported that trypsin inhibitors, lectins and tannins are naturally present in raw peas (Le Guen et al., 1995) and barley (Salunkhe et al., 1990). Tannins can reduce adsorption of AA in the gut (Jasman, 1993). Trypsin inhibitors decrease proteolysis and increase endogenous nitrogen secretion (Clarke and Wiseman, 2005). Finally lectins can reduce digestion and utilization of CP and AA (Van Nevel et al., 1998).

Le Guen et al. (1995) showed that the presence of ANF can affect performance. The authors found a reduction in ADG (from 290 to 239 g/d) in piglets fed diets containing whole pea (25% w/w) than those fed diets containing pea protein isolate (18% w/w). This isolate is commercially obtained by alkaline extraction of proteins from peas, followed by acidic precipitation, and it is free of ANF (Sumner et al., 1981). In their study, Le Guen et al. (1995), reported that the pea protein isolate processed from 2 different cultivars of pea were 50 and 71% lower in trypsin inhibitor activities and 60 and 56% lower in lectins concentration than the respective whole peas from which they were produced. In a previous study; Jansman and Van Diepen (2005) evaluated 2 diets (a corn-SBM and a wheat-barley) and 4 SID TLRs (14.7, 17.6, 20.5 and 23.4%) in nursery pigs. The authors found an increase in ADG and ADFI with the highest SID TLR. They also found a better performance for the piglets fed the wheat-barley diet, suggesting an increase in the SID TLR in diets made from corn and SBM. More recently, Jansman et al. (2010) also found that piglets fed a wheat-barley diet had higher ADG, ADFI and GFR than those fed a corn-SBM. The authors could not find an obvious reason for this difference, since the nutritional content of both diets were fairly similar. They suspect that the differences could be

attributed to the diverse palatability of the ingredients used (or the combinations of ingredients), or differences in the actual AID values compared to the calculated ones. The authors also suggested that the requirement of Trp is not dependant on diet composition.

The US diet had lower ($P < 0.10$) average PUN values than the WC diet (Table 3.8). Coma et al. (1995) mentioned that quality of the protein affects PUN; in this sense, worse protein quality will increase PUN and better protein quality will decrease PUN. This can also be a reflection of the presence of ANF on the WC diet.

Surprisingly, there was no effect ($P > 0.10$) of the TLR or the interaction of diet type and TLR in any parameters evaluated (for the performance trial and the N balance study; Table 3.7 and 3.8). The only exception was the low TLR diet which had a lower ($P < 0.10$) final PUN (1.68 ± 0.19 mmol/l) than the high TLR diet (1.94 ± 0.45 mmol/l). These results are in contradiction to previous studies (Burgoon et al., 1992; Henry et al; 1992, 1996; Eder et al., 2001) which found a clear response of pigs to Trp supplementation.

An explanation for this can be that the levels of Lys (1.48 ± 0.02% on average, total AA basis; or 1.34 ± 0.02% on average, SID basis) were above the pig requirement. Lys levels were set following the guidelines from Evonik-Degussa (1.35% SID). It has been pointed out that in dose response trials, an excess in Lys supplementation (over the requirement) can cause an underestimation of the optimal TLR (Van Cauwenberghe and Relandeau, 2000) because protein or other non essential AA will become limiting (Susenbeth, 2006). Barea et al. (2009) suggested that in order to find the adequate ratio of an AA relative to Lys, the latter should be the second factor limiting performance in the study. Ideally, Lys requirement should

be set at 90 or 95% of the requirement. Setting the Lys level lower than that can compromise performance (Van Cauwenberghe and Relandeau, 2000). Literature shows different values for SID Lys requirement for weaned piglets. The NRC (1998) recommends a level of SID Lys of 1.19%. Yi et al. (2006) found a SID Lys requirement of 1.32%. More recently Khendall et al. (2008) recommended a SID Lys level of 1.30%.

The vast difference in the literature about the optimal Lys level for nursery pigs and the lack of effect of Trp supplementation in this study can lead to the conclusion that the levels of Lys were set above the pig's requirement.

3.5.2 Experiment 2

The purpose of this experiment was to evaluate the effect of CP level on the TLR. For this experiment the US type of diet was selected because its better ADG, BW and GFR obtained in Experiment 1. Two levels of CP were chosen to be tested: 20% (high) vs. 17% (low); and two SID TLR were tested: 16% vs. 20%. The analyzed CP values of the diets (21.05 ± 0.08% and 19.03 ± 0.07%; Table 3.6) were higher than the proposed ones. The reason behind this could be that the analysis underestimated the CP content of SDBP. NRC (1998) indicates a CP of 78% for SDBP; however, the analysis showed a value of 72.25%. The rest of the N-containing ingredients were very close to the published values. The analysed SID Lys level (1.28 ± 0.03%) was somewhat lower than the target (1.35%). It was important to keep the same Lys level as Experiment 1 for comparison purposes. The analyzed SID Trp levels (0.23 ± 0.01% and 0.29 ± 0.02%) were in close agreement to the proposed ones (0.22% and 0.28%).

However, since the analyzed SID Lys level was a little bit lower, the analysed SID TLR were higher than the proposed ones (18.01 ± 0.05 vs. 15.95 ± 0.05% for the lower ratio; and 22.27 ± 2.24% vs. 20.00 ± 0.04% for the higher ratio).

While in Experiment 1 dietary treatments were offered as mash; in Experiment 2 diets were offered as pellet to reduce feed wastage (it easier to adjust the feeders to avoid waste using pellets instead of mash) and to reduce the work associated with weighing of feed refusal.

Originally the whole experiment was planned to be done in one batch (i.e. 96 piglets at once); however, the pig supplier from Experiment 1 was unable to deliver the pigs. A new supplier was found but they could not provide the whole number of animals at once, hence the experiment was divided into two batches (i.e. 48 piglets each) to meet the supplier output volumes. There were some differences between batches ($P < 0.10$) among the performance parameters evaluated. Pigs from batch 2 had heavier initial BW (7.17 ± 0.07 kg vs. 6.63 ± 0.07 kg) and final BW (16.77 ± 0.80 kg vs. 15.52 ± 1.22 kg) than those from Batch 1. The difference on final BW between batches was a consequence of its difference in arrival BW because both batches were fed the same starter diet for the same amount of time (2 d). In addition, batch 1 had a higher ($P < 0.01$) initial PUN (4.36 ± 1.35 mmol/l) than batch 2 (2.60 ± 0.70 mmol/l; data not shown. The lower initial PUN value for batch 2 suggests a better N utilization of the starter diet than the pigs from batch one (Coma et al., 1995). This was also supported by the N balance data in which the pigs from batch 2 retained more ($P < 0.01$) N (79.02 ± 2.41%) than those from batch 1 (69.28 ± 7.82%).

There was no effect ($P > 0.10$) of Trp supplementation on any of the parameters evaluated. These results are in disagreement with previous work. Jansman et al.

(2000) evaluated the relationship between levels of branched chain amino acids (BCAA; Leu, Ile and Val) with Trp in diets for nursery pigs. They formulated 2 Trp-deficient diets (0.16% SID Trp) with 2 different Trp-to-BCAA ratios. The authors evaluated two levels of Trp supplementation (0.20 or 0.24 % SID Trp). They found that increasing the levels of Trp improved ADG, ADFI and GFR. The maximum performance value was observed for the pigs fed the highest Trp-to-BCAA ratio.

The authors concluded that ADFI is increased by dietary high levels of Trp especially in low protein diets because those diets have lower levels of BCAA (i.e. higher Trp-to-BCAA). The reason behind is that BCAA also compete with Trp for the passage through the blood-brain-barrier, a higher Trp-to-BCAA ratio will increase the availability of Trp for serotonin synthesis and improve feed intake (Henry et al., 1996). Similarly, Guzik et al. (2002) also found a linear increase of ADFI, ADG and GFR in phase I, II and III pigs when Trp supplementation was increased. Finally, in a 3-wk study, Boomgaardt and Baker (1973) tested 3 levels of CP (10, 14 and 18%) and 5 levels of Trp (0.33, 0.50, 0.67, 0.83 and 1.00 % of CP). They found a linear and quadratic increase in ADFI and GFR when the Trp supplementation was increased.

The high-CP diet had higher ADG and better GFR than the low-CP diet; however, it was not reflected on heavier final BW of the high-CP diet. Similar results were obtained in previous work. Boomgaardt and Baker (1973) found an increase in ADG (in all the Trp supplementation levels) when CP was increased from 10% to 18% on nursery pigs. More recently, Le Bellego et al. (2002) found that growing pigs (from 27 to 100 kg BW) fed low-CP diets supplemented with AA had lower ADFI than those fed high-CP diets; however, there were no differences on net energy intake

or ADG. Kerr et al. (2003) suggested that CP levels had little or no effect over long periods if the levels of supplemented AA are adequate.

Final PUN and average PUN were affected by the CP content of the diet. The reduction of PUN with the low-CP diet can be a result of a more efficient N utilization (Coma et al., 1995). This is also supported by the N balance study where the low-CP diet had numerically higher N retention and lower N excretion (Table 3.10) suggesting that there was an excess of N on the diets. Indeed, the NRC (1998) recommends a level of CP of 20.9% for pigs in the BW range of 10 to 20 kg. The analyzed CP of the high-CP was 21.05% and that of the low-CP was 19.03% (Table 3.6). The excess of N on the high-CP (above the requirement) diet caused the increase in PUN.

As expected pigs, fed the high-CP diet ingested more N than those fed the low-CP diets. Clearly the high-CP diets contained more N (g/kg of diet), hence it is logical that pigs will consume more N than those fed the low-CP diets.

Similar to Experiment 1, we suspect that the levels of SID Lys of the diets (1.28 ± 0.03 on average) were above the pig's requirement even though the level was somewhat lower than in Experiment 1 (1.34 ± 0.02). Literature suggests SID Lys levels from 1.19% (NRC, 1998) to 1.32% (Yi et al., 2006). It can argue that the lack of effect of Trp supplementation may have been caused by the excess Lys which was not the second limiting AA in the study and possibly covered up the effect of Trp supplementation (Van Cauwenberghe and Relandeau, 2000). Another explanation for the lack of effect of Trp supplementation can be that the low TLR was not deficient in Trp. Guzik et al. (2002) found that the optimal SID Trp of nursery pigs should be 0.20% (from 6 to 10 kg BW) and 0.18% (from 10 to 15 kg BW). The NRC (1998)

suggests a level of SID Trp of 0.18% (17.82% SID TLR). The analysis of the diets showed that the low SID TLR was 18.01 ± 0.54% (0.22% SID Trp). Apparently those diets were not Trp-deficient. Susenbeth (2006) pointed out that in order to see an effect of Trp supplementation, the doses chosen should range from deficiency to excess. In this case both levels were theoretically above the Trp requirement.

To summarize, there was no effect of the TLR on any of the parameters evaluated. Similarly no effect of the interaction of TLR with CP levels was observed. Some of the reasons for this lack of effect are that the level of Lys was above requirement. In addition, apparently the low TLR diets were not deficient on Trp.

3.6 CONCLUSION

In both experiments there was a lack of effect of Trp supplementation or the interaction with CP and diet type on the performance and N balance parameters evaluated. The evidence suggests that the diets had a surplus of Lys hence the lack of effect of the variation of TLR. In experiment 1 the US diet had higher ($P < 0.10$) ADG, final BW, GFR and lower ($P < 0.10$) PUN than the WC diet. In Experiment 2, the high-CP diet had higher ($P < 0.01$) ADG, GFR and PUN than the low-CP diet. Pigs fed the high-CP diet consumed and excreted more ($P < 0.10$) N (g/d) than the low-CP diets.

The evidence suggests that further research is needed to evaluate the effect of CP level and dietary composition on Trp supplementation. From these results we can suggest a pre-determination of the Lys requirement, to ensure that its level is kept between 90 and 95% of the requirement, in order to visualise the real effect of Trp

supplementation. In addition, ensuring a Trp-deficient diet should be a key aspect on future Trp dose-response studies.

CHAPTER 4

MANUSCRIPT TWO

Lysine requirement of early weaned pigs fed a wheat-barley-based diet. Borgesa, G. [1], R. L. Payne [2], and C. M. Nyachoti [1]

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4.1 ABSTRACT

Lys is the first limiting amino acid (AA) in nursery diets. The NRC (1998) states a SID Lys requirement of 1.01% for 10 to 20 kg pigs; however, there is evidence that this requirement is lower than optimal. So, the objective of this 15-d study was to determine the SID Lys requirement of early weaned pigs. Yorkshire x Landrace pigs (n=72, initial BW: 8.73 ± 1.13 kg) were blocked by BW and gender, group housed (3 pigs/pen), and allotted to 6 dietary treatments (4 pens/diet). Supplemental Lys was added to a wheat-barley-Lys-deficient basal diet (0.89% SID Lys) in order to create graded levels of SID Lys (0.89, 0.98, 1.21, 1.27, 1.32 and 1.33%). Pigs were allowed *ad libitum* access to feed and water. Average daily feed intake (ADFI), average daily gain (ADG), gain-to-feed ratio (GFR) and plasma urea nitrogen (PUN) were measured every 5 d and used as response criteria. Increasing SID Lys had no effect on ADFI (P = 0.23, 0.56 kg/d on average), ADG (P = 0.68, 0.34 kg/d on average), GFR (P = 0.38, 0.60 kg BW gain/kg feed on average) or pooled PUN (P = 0.23, 3.85 mmol/l on average). No linear increase ($P > 0.10$) was observed on ADG but was observed ($P < 0.10$) on ADFI, GFR and pooled PUN. GFR and pooled PUN were analyzed using the non-linear (NLIN) procedure (broken-line analysis) of SAS to determine the Lys requirement. As SID Lys level increased from 0.89 to 1.33%, the GFR increased (from 0.57 to 0.62 BW gain/kg feed) linearly (P = 0.03). Break point analysis suggested a SID Lys requirement of 1.03 ± 0.06% (P = 0.01; R^2 = 0.36). PUN decreased (from 5.28 to 3.45 mmol/l) linearly (P = 0.01) and quadraticaly (P = 0.08). Break point analysis yielded a SID Lys requirement of 1.01 ± 0.04% (P = 0.01; R^2 = 0.36). Taking the average of the GFR and pooled PUN estimates, the SID Lys requirement for early weaned pigs fed wheat-barley based

diets was estimated at 1.02% (1.21% on a total amino acid basis). These results are in close agreement with the NRC (1998) recommendations for nursery pigs, which some authors considered lower than optimal.

Key Words: lysine, requirements, weaned pigs

4.2 INTRODUCTION

Lys is usually the first limiting AA in nursery pigs (Gaines et al., 2005). With today's new and improved genetic; there is a need to determine the optimal Lys requirement (Yi et al., 2006) to maximize growth and efficiency. In addition, when implementing dose response trial of other essential AA, the levels of Lys should be kept at 90 or 95% of the requirement to ensure accuracy of the study (Van Cauwenberghe and Relandeau, 2000).

There is a lot of discrepancy regarding the optimal Lys requirement of nursery pigs. NRC (1998) recommends a SID Lys of 1.01% and some authors agree with this recommendation (Martinez and Knabe, 1990; Roth et al., 1999). On the other hand, there are many studies that recommend higher levels of SID Lys. For example, Smith et al. (1999) suggested a Lys level of 1.30%, and Van Lunen and Cole (1998) recommended a Lys level of 1.50%. More recently, levels of 1.23% (Urynek and Buraczewska, 2003) and 1.35% (Kendall et al., 2008) have been suggested for nursery pigs. Many factors (such as breed, health status, statistical method used, etc) are responsible for such variations (Kendall et al., 2008).

Even though there are many statistical methods used to determine the optimal requirement of AA, the broken-line analysis is preferred because it is relatively

simple, shows the response of an AA for the whole range of supplementation, and provides the requirement as the break point of the two lines (Robbins et al., 2006).

Previous research within our group failed to show an effect of Trp supplementation on nursery pigs. The main reason was an excess of Lys (above requirement). In order to determine the optimal SID TLR for weaned pigs, we need to know first the requirement of the first limiting AA (Susenbeth and Lucanus, 2005). Hence, the objective of this study was to determine the SID Lys requirement of weaned pigs (Yorkshire x Landrace; Iceman Genetics, St. Eustache, MB, Canada) using the broken-line analysis.

4.3 MATERIALS AND METHODS

Timeline

Pigs arrived at the T.K. Cheung Center for Animal Research (TKCCAR) on January 3, 2008. After 4 d of adaptation the trial started on January 7, 2008 and ended on January 22, 2008 (a total of 15 d).

Animals and Diets

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee (Protocol Nº F05-024/2). Pigs were cared for following the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

For this study, seventy-two Yorkshire x Landrace piglets (36 barrows and 36 gilts) weaned at 17 ± 1 d were obtained from Iceman Genetics (St. Eustache, MB, Canada). After 4 d of adaptation to a commercial starter diet, 4 spare pigs (2 barrows and 2 gilts) were removed based on their BW (the heaviest and lightest from each

gender), the average initial BW was 8.73 ± 1.13 kg. Pigs were weighed and blocked on the basis of BW and sex and randomly allotted into groups of three pigs per pen (same gender). Each pen was considered the experimental unit. There were a total of 24 experimental units.

Four experimental units (2 of each gender) were assigned to one of the six dietary treatments. Before mixing all the N-containing ingredients were sampled and sent to Degussa Canada (Burlington, ON, Canada) for CP and AA content analysis (Table 4.1).

The experimental basal diets were mixed at the University of Manitoba feed mill. All the ingredients were obtained from local suppliers as per normal procedure. Wheat, pea and barley were coarsely ground (~800µm) prior to mixing the diets. Corn gluten feed (CGF) was included in the formulation to ensure low levels of Trp. Two basal wheat-barley diets were formulated: 1) a Lys deficient diet (SID Lys 0.89%) and 2) a Lys surplus diet (same Lys deficient diet plus supplemental Lys to achieve a SID Lys level of 1.33%). At the TKCCAR the other 4 diets were obtained by combining different amounts of the basal diets (Table 4.2) in order to obtain graded levels of SID Lys (i.e. 0.89, 0.98, 1.21, 1.27, 1.32 and 1.33%). The combined diets (batch of 150 kg each) were mixed on a horizontal mixer for 10 min. Diets were formulated to differ only in their Lys content. All other nutrients were supplied in amounts meeting or exceeding NRC (1998) recommendations and using Rademacher et al. (2000) SID coefficients (Table 4.3). To accomplish proper AA mixing of the basal diets, the crystalline AA were pre- mixed with 15 kg of wheat (a portion of the diet formulation). The wheat-AA premix was mixed with the rest of the ingredients.

Table 4.1. Analyzed [a] CP and AA content of N-containing ingredients (as-fed basis) for Experiment 3 and Experiment 4.

	N – Content Ingredient						
Nutrient, g/kg	Peas	Barley	Wheat	SBM	CGF	Whey	SDBP
Dry Matter	882.8	893.8	871.3	890.5	898.2	973.6	939.7
Crude Protein	196.0	104.5	120.9	465.3	227.0	123.3	793.4
Methionine	1.9	1.8	1.9	6.5	4.2	1.8	6.9
Cystine	2.9	2.3	2.7	7.3	4.3	2.5	23.1
Methionine + Cystine	4.7	4.0	4.6	13.7	8.5	4.3	30.0
Lysine	14.2	3.8	3.4	29.2	8.1	9.5	70.7
Threonine	7.4	3.5	3.5	18.4	8.1	8.3	44.9
Tryptophan	1.8	1.3	1.5	6.4	1.8	1.9	14.0
Arginine	17.5	5.4	5.8	34.4	12.8	2.5	44.8
Isoleucine	8.2	3.5	3.9	20.9	7.6	6.7	26.9
Leucine	14.0	7.0	7.9	35.4	19.3	10.9	78.2
Valine	9.2	5.0	5.0	22.3	11.4	6.2	51.2
Histidine	4.6	2.3	2.7	12.5	6.8	2.2	28.3
Phenylalanine	9.6	5.0	5.3	23.6	8.8	3.4	44.4
Glycine					11.0	2.3	29.2
Serine					9.5	5.9	44.6
Proline					16.3	6.7	42.2
Alanine					15.3	5.5	44.3
Aspartic acid					14.3	12.0	78.2
Glutamic acid					34.3	20.0	108.9

[a] Peas, barley, wheat and SBM were analyzed using NIR spectroscopy as described by Fontaine et al. (2002, 2004). CGF, dried whey and SDBP were analyzed using wet chemistry following the guidelines of Commission Directive (1998, 2000).

Pigs were group housed in floor pens (length, 1.8 m; x width 1.2 m; x height, 0.9 m height) with plastic-covered expanded metal sheet flooring in a temperature controlled room (starting at 30ºC in wk one; temperature was reduced 1ºC/wk) with a 12 h light/dark cycle. Pigs were allowed *ad libitum* access to feed and water through a nipple drinker and a feeder. Diets were offered as mash. The performance trial was conducted over a 15-d period. Feed disappearance and pig live weight were monitored every five days and the two parameters used to calculate ADG, ADFI and

Table 4.2. Composition of experimental diets [a] (as-fed basis) used in Experiment 3.

Ingredient	Standardized ileal digestible lysine level (%) [a]					
	0.89	0.98	1.21	1.27	1.32	1.33
Barley	19.09	19.09	19.09	19.09	19.09	19.09
Soybean meal	4.00	4.00	4.00	4.00	4.00	4.00
Corn gluten feed	31.96	31.77	31.43	31.28	31.14	30.99
Peas	13.18	13.18	13.18	13.18	13.18	13.18
Wheat	12.50	12.50	12.50	12.50	12.50	12.50
Spray dried blood plasma	4.80	4.80	4.80	4.80	4.80	4.80
Dried whey	6.50	6.50	6.50	6.50	6.50	6.50
Vegetable oil	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.08	1.08	1.07	1.07	1.06	1.06
Biophos	0.50	0.51	0.52	0.53	0.53	0.54
Vitamin / mineral premix [b]	1.00	1.00	1.00	1.00	1.00	1.00
Biolys	0.00	0.19	0.51	0.65	0.79	0.93
DL-methionine	0.18	0.18	0.18	0.18	0.18	0.18
L-threonine	0.00	0.00	0.01	0.01	0.01	0.01
L-tryptophan	0.04	0.04	0.04	0.04	0.04	0.04
L-isoleucine	0.13	0.13	0.13	0.13	0.13	0.13
L-valine	0.04	0.04	0.05	0.05	0.05	0.05

[a] Diet A: Lys deficient (0.89% SID Lys); Diet F: Lys surplus (1.33% SID Lys); Diet B: mixture of 80% Diet A and 20% Diet F (0.98% SID Lys); Diet C: mixture of 45% of Diet A and 55% of Diet F (SID Lys 1.21%); Diet D: mixture of 30% of Diet A and 70% of Diet F (1.27% SID Lys); and Diet E: mixture of 15% of Diet A and 85% of Diet F (1.3% SID Lys).

[b] Providing the following per kilogram of diet: vitamin A, 8255 IU; vitamin D3, 1000 IU; vitamin E, 20 IU; vitamin K, 1.5 mg; riboflavin, 7.5 mg; niacin, 30 mg; vitamin B12, 25 µg; pyridoxine, 4.5 mg; biotin, 200 µg; folic acid, 1 mg; thiamin, 4 mg; choline, 781 mg.; copper, 10 mg; iodine, 0.6 mg; iron, 130 mg; manganese, 40 mg; selenium, 0.3 mg; zinc, 130 mg.

GFR. Every 5 d, blood samples were obtained from one pig per pen for the determination of PUN.

Sample preparation and chemical analyses

Samples of diets and N-containing ingredients (i.e. peas, barley, wheat, SBM, CGF, dried whey and SDBP) were prepared as described in section 3.3.1.

For the PUN samples, 10 ml of blood was collected from one pig per pen via jugular vein puncture into sodium-heparin-vacuntainer-tubes (Becton Dickinson,

Table 4.3. Nutrient Content of Experimental Diets (as-fed basis) of Experiment 3.

	Standardized ileal digestible lysine level (%)					
Item	0.89	0.98	1.21	1.27	1.32	1.33
Dry Matter, g/kg [a]	915.3	910.2	909.2	908.1	908.8	915.6
Ether extract, g/kg [b]	69.1	69.0	68.9	68.9	68.9	68.8
CP, g/kg [a]	209.2	208.0	212.0	205.5	206.8	207.8
CP, % [a]	20.9	20.8	21.2	20.6	20.7	20.8
Crude fiber, g/kg [b]	64.2	64.0	63.6	63.5	63.3	63.1
Digestible Energy, MJ/kg [a]	14.1	14.1	14.0	14.0	14.0	14.0
Net Energy, MJ/kg [b]	9.0	9.0	10.0	10.0	10.0	10.0
Amino acids, g/kg [a]						
Methionine	4.8	4.8	4.8	4.9	4.9	4.7
Cystine	4.1	4.2	4.2	4.2	4.2	4.2
Methionine + Cystine	9.0	9.0	9.0	9.2	9.2	8.8
Lysine	**10.8**	**11.7**	**13.9**	**14.6**	**15.3**	**15.2**
Threonine	8.3	8.5	8.5	8.4	8.5	8.2
Tryptophan	2.7	2.6	2.7	2.7	2.8	2.7
Arginine	12.2	12.5	12.4	12.3	12.4	12.3
Isoleucine	8.6	8.7	8.7	8.7	8.7	9.0
Leucine	16.5	16.8	16.7	16.6	16.6	16.4
Valine	10.9	11.2	11.3	11.2	11.2	11.1
Histidine	5.5	5.5	5.5	5.4	5.5	5.4
Phenylalanine	9.4	9.6	9.5	9.4	9.5	9.5
Glycine	8.5	8.7	8.6	8.5	8.7	8.5
Serine	9.7	9.9	9.8	9.7	9.8	9.6
Proline	13.9	14.1	14.0	13.7	13.7	13.6
Alanine	10.8	11.1	11.0	11.0	11.0	10.8
Aspartic acid	16.4	16.8	16.8	16.5	16.6	16.5
Glutamic acid	34.4	34.8	34.7	34.5	34.4	34.5
SID Lysine, g/kg [a]	8.9	9.8	12.0	12.7	13.4	13.4

[a] Diets were analyzed using wet chemistry following the guidelines of Commission Directive (1998, 2000).
[b] Calculated values.

Rutherford, NJ) on days 0, 5, 10 and 15. Blood samples were processed and analysed as described in section 3.3.1.

Statistical analysis

ADFI, ADG and GFR were calculated as described in section 3.3.1. Pooled PUN was calculated as the average of the PUN measurements done in days 10 and 15.

Data residuals and normality were tested as described in section 3.3.1.

Data was analyzed as a completely randomized design using Proc GLM of SAS (SAS Inst., Inc., Cary, NC). When a significant F-value ($P < 0.10$) was indicated by the ANOVA, the Scheffe test was used to compare treatments means. The model used was $y_{ij} = \mu + d_i + e_{ij}$; where y_{ij} = initial BW, final BW, ADG, ADFI, GFR, initial PUN, Final PUN or pooled PUN of the j'th pen of the i'th SID Lys level; μ = population mean; d_i = main effect of the i'th SID Lys level; and e_{ij} = residual error of the j'th pen of the i'th SID Lys level. Since each treatment had 2 female and 2 male pens, gender was considered a cofactor on the ANOVA.

Linear and quadratic response were evaluated for ADFI, ADG, GFR and averaged PUN using orthogonal polynomials generated through Proc IML procedures of SAS (SAS Inst., Inc., Cary, NC) for unequally spaced treatment structure. A level of significance of 10% ($P < 0.10$) was used.

To determine the optimal SID Lys level GFR and PUN were subjected to a broken-line analysis (Robbins et al., 2006). GFR and PUN were analyzed using the Proc NLIN of SAS (SAS Inst., Inc., Cary, NC), the model used was: Y = L + U*z1. Where Y = GFR or PUN; L = asymptote; U = slope of the curve; z1 = (Lysine < Requirement)*(Requirement-Lysine). The coefficient of determination (R^2) was calculated using the following equation (Robbins et al., 2006): R^2 = [(Corrected Sum of Squares) – (Error Sum of Squares)] \ (Corrected Sum of Squares).

4.4 RESULTS

All animals remained healthy during the experimental period. There were no differences ($P > 0.10$) on the initial BW (8.73 ± 0.14 kg) or the final BW (13.71 ± 0.28 kg) due to SID Lys content. ADFI was similar for the six treatments (557± 39 g/d). There were no differences ($P > 0.10$) due to Lys content of the diet on ADG (0.33 ± 0.02 kg/d) and GFR (0.60 ± 0.02 kg BW/kg feed). With the exception of initial BW ($P = 0.06$; on average males were 0.50 kg heavier than females; data not shown), there were no effects of gender ($P > 0.10$) on any of the performance parameters evaluated (Table 4.4).

There were no differences ($P > 0.10$) due to Lys content of the diet on the baseline PUN (4.66 ± 0.42 mmol/l) or the final PUN (3.87 ± 0.72 mmol/l). Pigs fed the 1.21% SID-Lys-diet had lower ($P < 0.10$) pooled PUN than those fed the 0.89% SID-Lys diet. There were no effect of gender ($P > 0.10$) on any of the PUN parameters evaluated (Table 4.5).

There were no linear or quadratic effects ($P > 0.10$) due to the dietary Lys content on initial and final BW and ADG. There was a linear ($P = 0.07$) but not quadratic ($P > 0.10$) increase in ADFI when the Lys level was increased. There was also a linear ($P = 0.08$) but not quadratic ($P > 0.10$) improvement in GFR when the Lys level of the diet was increased (Table 4.4; Figure 4.1). There were no linear or quadratic effects ($P > 0.10$) on the baseline PUN measurement. A linear ($P = 0.03$) but not quadratic ($P > 0.10$) decrease on the final PUN measurement was observed when the Lys content of the diet was increased. Finally, the pooled PUN measurement was decreased both linearly ($P = 0.01$) and quadraticly ($P = 0.08$) when Lys levels on the diets were increased (Table 4.5; Figure 4.3).

Table 4.4.- Performance of early-weaned piglets fed a wheat-barley based diet with six levels of SID Lys.

Item	Standardized ileal digestible lysine level (%)						SEM	*P* Values		Contrast [c]	
	0.89	0.98	1.21	1.27	1.32	1.33		SIDlys [a]	Gender [b]	Linear	Quadratic
Initial BW [d], kg	8.84	8.85	8.46	8.80	8.71	8.72	0.308	0.951	0.060	0.637	0.651
Final BW [d], kg	14.14	13.71	13.53	13.75	13.30	13.84	0.451	0.843	0.256	0.396	0.602
ADFI [e], g/d	627	545	546	550	510	561	31.14	0.228	0.860	0.070	0.295
ADG [f], kg/d	0.354	0.326	0.341	0.333	0.309	0.345	0.020	0.678	0.913	0.497	0.779
GFR [g]	0.566	0.600	0.623	0.605	0.606	0.615	0.019	0.381	0.607	0.081	0.225
n [h]	4	4	4	4	4	4					

[a] Effect of the SID Lys level of the diet.
[b] Effect of pig gender
[c] Linear or quadratic effect of graded levels of SID Lys of the diet.
[d] Body weight.
[e] Average daily feed intake
[f] Average daily gain.
[g] Gain-to-feed ratio (kg feed / kg BW).
[h] Observations per treatment (each observation is the average of three pigs).

Table 4.5.- Plasma urea N in early-weaned piglets fed a wheat-barley based diet with six levels of SID Lys.

Item	Standardized ileal digestible lysine level (%)						SEM	*P* Values		Contrast [e]	
	0.89	0.98	1.21	1.27	1.32	1.33		SIDlys [c]	Gender [d]	Linear	Quadratic
PUN, mmol/l											
Baseline	4.90	4.45	4.15	4.30	4.95	5.20	0.504	0.644	0.873	0.829	0.105
Final Measure	5.18	3.85	3.20	4.13	3.50	3.38	0.492	0.109	0.759	0.027	0.186
Pooled Measure [f]	5.28 [b]	3.89 [ab]	3.19 [a]	3.70 [ab]	3.59 [ab]	3.45 [ab]	0.466	0.071	0.974	0.012	0.084
n [g]	4	4	4	4	4	4					

[a,b] Means with different superscript are statistically different ($P < 0.10$)
[c] Effect of the standardized ileal digestible lysine level of the diet.
[d] Effect of pig gender
[e] Linear or quadratic effect of graded levels of SID Lys of the diet.
[f] Means are the average of measurements done on day 10 and 15.
[g] Observations per treatment

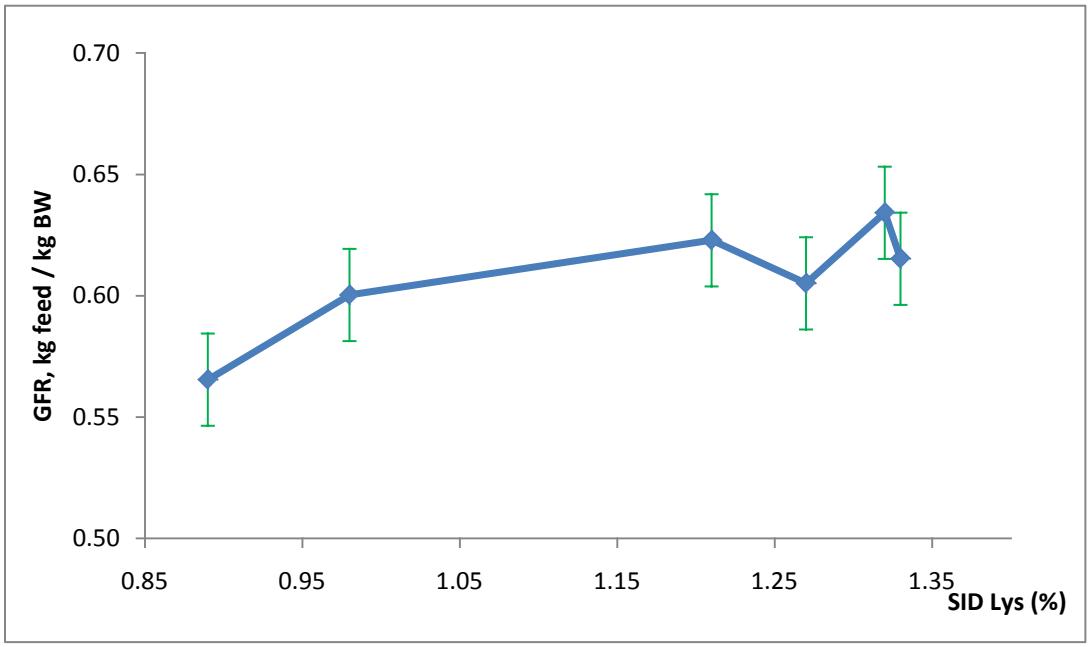

Figure 4.1. GFR response of nursery pigs fed a wheat-barley diet with graded levels of SID Lys (Linear: *P* = 0.08; Quadratic: *P* = 0.23)

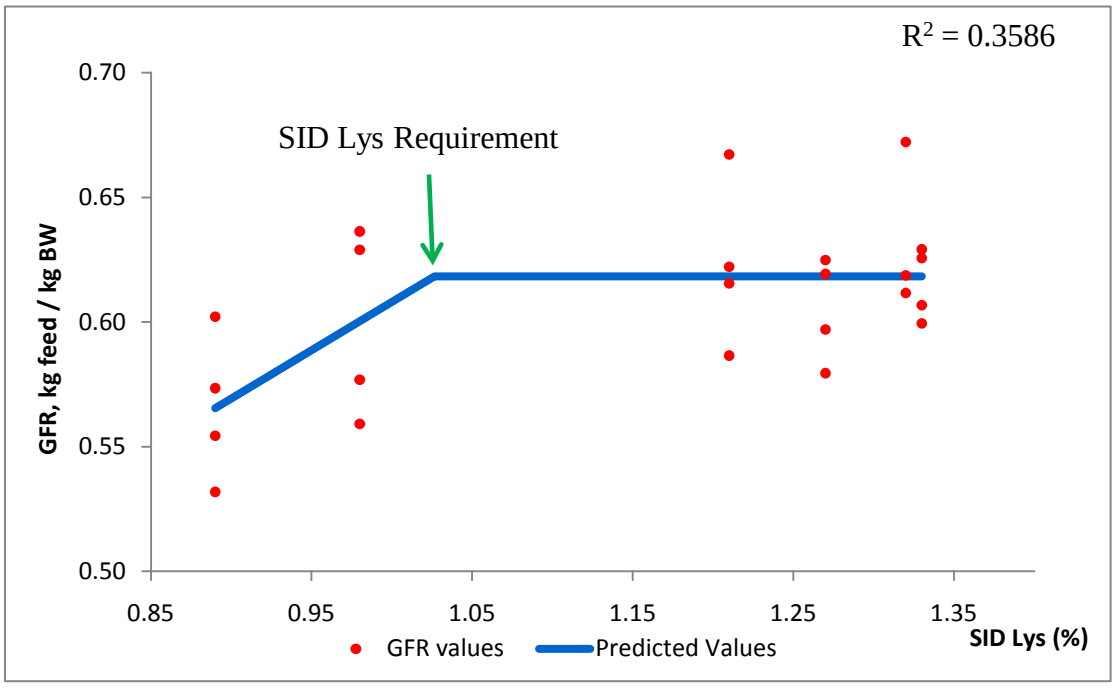

Figure 4.2. Determination of SID Lys requirement using the broken-line analysis and GFR as a response criteria.

Equation line : Pred GFR = L + U * (Z1). Where: Pred GFR = predicted value of GFR ; L = Asymptote = 0.618 ± 0.007; U = Slope = -0.388 ± 0.223; Z1= (X < SID Lys) * (Requirement – SID Lys). Final SID Lys Req = 1.03 ± 0.062 (*P* = 0.01)

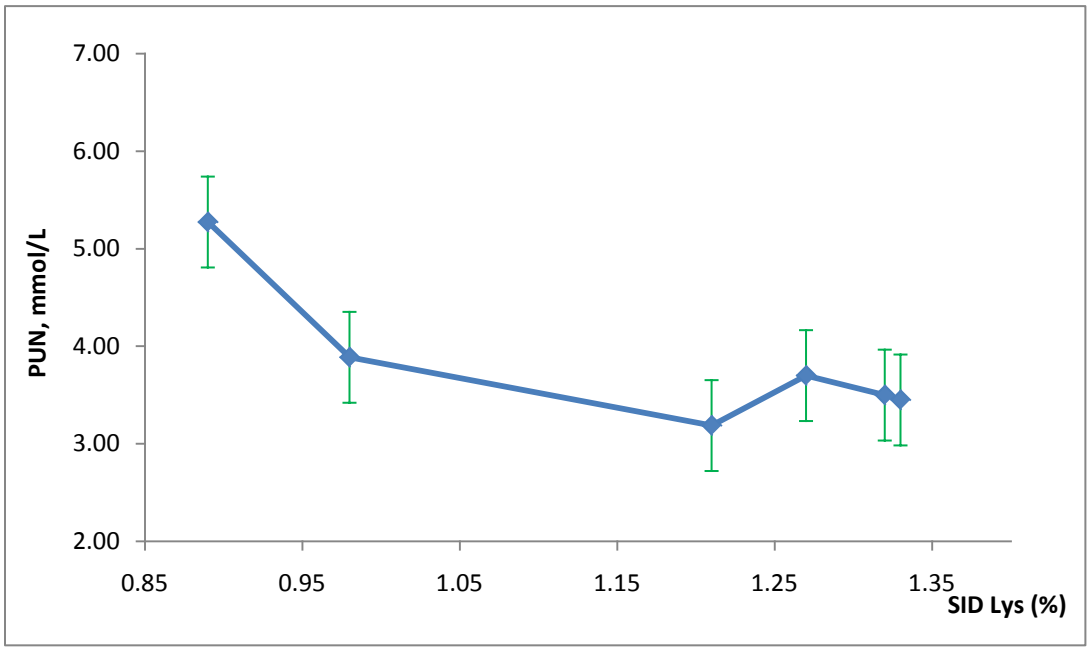

Figure 4.3. PUN response of nursery pigs fed a wheat-barley diet with graded levels of SID Lys (Linear: *P* = 0.01; Quadratic: *P* = 0.08).

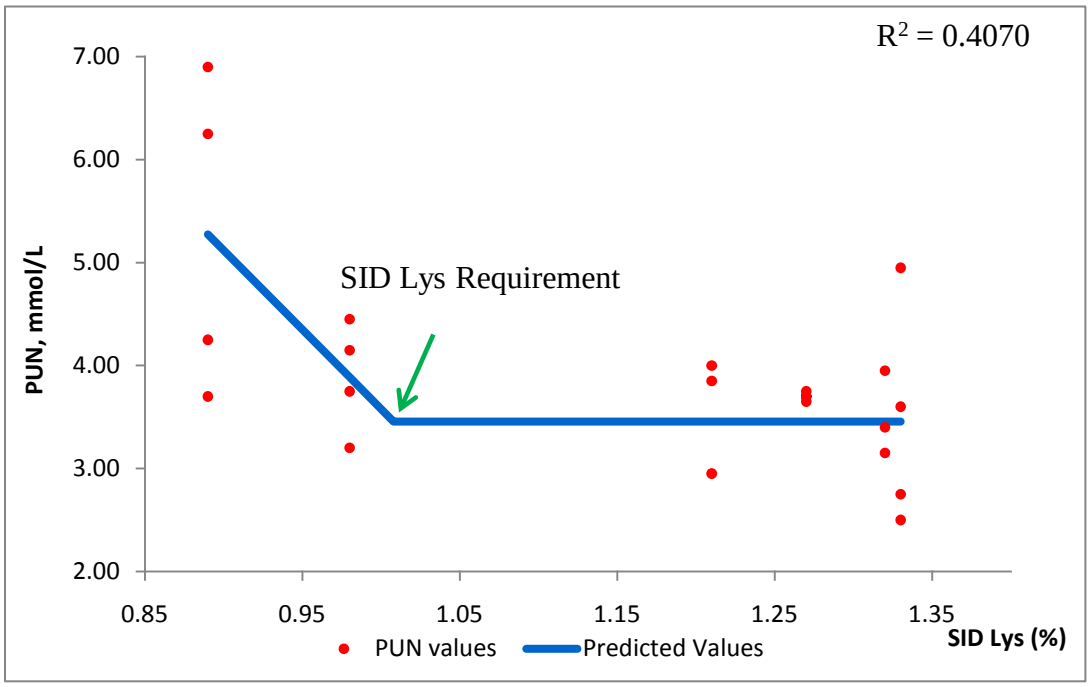

Figure 4.4. Determination of SID Lys requirement using the broken-line analysis and pooled PUN as a response criteria.

Equation line : Pred PUN = L + U * (Z1). Where: Pred PUN = predicted value of PUN; L = Asymptote = 3.457 ± 0.225; U = Slope = 15.417 ± 6.855; Z1= (SID Lys < Requirement) * (Requirement – SID Lys). Final SID Lys Req = 1.01 ± 0.041 (*P* < 0.01)

Using GFR as response criteria and subjecting it to the broken-line analysis, we determined a SID Lys requirement of 1.03 ± 0.06% (1.22 ± 0.06% total AA; $P = 0.01$; $R^2 = 0.36$; Figure 4.2). Similarly, using pooled PUN as the response criteria, the SID Lys requirement was 1.01 ± 0.04% (1.20 ± 0.04% total AA; $P = 0.01$; $R^2 = 0.41$; Figure 4.4). Taking the average of both response criteria, the SID Lys requirement of weaned pigs fed a wheat-barley diet is 1.02 ± 0.01% (1.21 ± 0.01% total AA).

4.5 DISCUSSION

The purpose of this study was to determine the SID Lys requirement of early-weaned pigs Yorkshire x Landrace fed a wheat-barley diet. And use the information obtained to determine the optimal SID TLR of early weaned pigs in a subsequent experiment (Chapter 5). This particular diet type was chosen because it is usually one of the most common diets used in Western Canada due to the easy availability of both cereals for animal feed. Indeed, wheat and barley are the cereals most produced in Manitoba. Together they represent almost 35% of the total crop production of the province (MAFRI, 2009). Since the same breed of pigs and diet type were going to be used in the subsequent experiment (i.e. determination of the optimal SID TLR; Chapter 5), CGF was added to the diets to ensure low levels of Trp (SID Trp content of CGF is close to 0.05%; NRC, 1998), and to be able to formulate a Trp-deficient basal diet for the subsequent experiment.

Originally the diets were planned to be pelletized, however the small final volume of each diet (150 kg) made it difficult to achieve a good quality of pellet (the feed mill pelletizer needs at least batches of 500 kg to ensure proper mixing and a good quality of pellets). Hence the decision was made to offer the diets as mash. The

targeted nutrient levels of the dietary treatments were in close agreement to the calculated ones. However, there were minor differences in the Lys and Trp levels. The proposed SID Lys levels were 0.89, 0.98, 1.15, 1.22, 1.29 and 1.36%. The analysed values were 0.89, 0.98, 1.21, 1.27, 1.32 and 1.33%. Similarly, the analyzed SID Trp level (0.23 ± 0.01%) was somewhat higher than the calculated value (0.19%). The calculated (20.22 ± 0.19%, on average) CP was very close to the analysed value (20.83 ± 0.21%, on average). The level of CP was set following NRC (1998) recommendations for 10 – 20 kg pig (Table 4.3).

Since there was no effect of SID Lys on the initial BW, there was no need to perform an analysis of covariance. In addition, all the variables of the study (i.e. ADG, ADFI, GFR and PUN) were checked to ensure they met the assumptions of the analysis of variance (Steel et al., 1997).

ADFI was similar to previous studies (530 g/d Burgoon et al., 1992; 553 g/d, Han et al., 1993; 559 ± 54 g/d, Eder et al., 2001). It was also in close agreement to the NRC (1998) reported values for feed intake (Table 4.4). However, it was lower than more recent studies. Yi et al. (2006) tested 5 graded levels of SID Lys (from 1.10 to 1.50%) in a corn-SBM diet. The authors used 662 nursery pigs (initial BW 12.20 ± 0.18 kg; final BW 24.24 ± 0.29 kg) for the study and ADFI was 864 ± 1 g/d. The higher ADFI was due to the higher initial BW of that study compared to this one (12.20 ± 0.18 vs. 8.73 ± 0.14 kg) and the longer duration of that trial (21 vs. 15 d). A more recent study (Kendall et al., 2008) tested 6 levels of SID Lys (from 1.05 to 1.40%) on a corn-SBM diet. The authors also found higher ADFI (740 g/d) during the first 14 d of trial. The authors just used barrows and their initial BW was also higher (11.44 ± 0.09 kg vs. 8.73 ± 0.14 kg). It is worth to mention that both studies

were performed by the same research group and also both studies used the commercial line of pigs (Dalland x PIC Camborough 22). It has been shown that the Dalland x PIC Camborough 22 commercial line had high protein deposition rates (Yi et al., 2006; Kendall et al., 2008). This can explain the better performance (higher ADG and GFR) of those pigs compared to our crossbred.

Table 4.4 also shows the lack of linear or quadratic effect of the graded levels of SID Lys of the diet on the performance parameters evaluated. The only exception were ADFI and GFR which were linearly increased ($P < 0.10$) when SID Lys level of the diet was increased. Previous work did not find an effect ($P > 0.10$) of crystalline Lys supplementation on feed intake (Urynek and Buraczewska, 2003; Yi et al., 2006) perhaps because the authors increased the level of Lys by increasing CP levels. The latter created an excess of protein and a need to deaminate the surplus protein. Thus affecting feed intake (Kendall et al., 2008). However, those authors (Urynek and Buraczewska, 2003; Yi et al., 2006; Kendall et al., 2008) found a linear increase in ADG as a result of increasing dietary Lys levels.

PUN can be used to accurately determine the Lys requirement in pigs because N metabolism changes quickly with changes in dietary AA (Coma et al., 1995). In this study, final PUN and pooled PUN were linearly and quadraticly (just for the pooled PUN) decreased when the Lys content of the diet was increased. These results are in agreement with previous studies. Dean et al. (2007) also found a linear reduction in PUN in nursery pigs when the SID Lys level was increased from 1.10 to 1.50%. Earlier, Nam and Aherne (1994) evaluated 4 Lys-to-digestible energy ratios (0.7, 0.8, 0.9 and 1.0 g Lys/MJ DE; and 13.3, 14.0 and 14.7 MJ DE) in weanling pigs fed a wheat-barley diet. Similar to our study, the authors found a linear decrease in PUN

from 10.51 to 8.01 mg/dL (3.75 to 2.86 mmol/L) as the Lys level increased from 0.7 to 0.9 g Lys/MJ DE (0.82 to 1.23% SID Lys). As mentioned before, PUN is considered a good measure to determine AA requirements (Coma et al., 1995; Guzik et al., 2002, 2005; Susenbeth, 2006) due to the fact that urea excretion is minimized when the requirement of the first limiting AA is met (Brown and Cline, 1974). Hence, the reduction of PUN will suggest a better utilization of N as a result of reaching the Lys requirement (Coma et al., 1995).

Proc NLIN of SAS (SAS Inst., Inc., Cary, NC) is one of the preferred procedures to determine the optimal AA requirements (Robbins et al., 2006). GFR and pooled PUN were chosen as the response criteria to be subjected to the non-linear broken line analysis for two reasons. The first one is that GFR is a performance criteria that combines feed consumption and daily gain into one parameter and PUN reflects the physiological response to changes in dietary AA intake (Coma et al., 1995). And secondly, there was a lack of linear or quadratic effects ($P > 0.10$) on the other parameters (with the exception of ADFI and final PUN) indicating that there were other factors affecting the piglet response.

One observation was removed from the broken line analysis for GFR because during the analysis of residuals using the PROC UNIVARIATE (SAS Inst., Inc., Cary, NC) it was considered an outlier. This observation came from a barrow pen from the 1.32% SID Lys level. This experimental unit had lower ADG (0.25 vs. 0.33 ± 0.01 kg/d) and ADFI (477 vs. 520 ± 32 g/d) compared to the average of the other 3 experimental unit of the treatment. As a result, the GFR of this pen (0.52 kg BW/ kg feed) was numerically lower than the average of the other treatment pens (0.63 ± 0.02 BW gain/kg feed). Even though this difference was not observed in the PUN broken-

line analysis, the experimental unit was still removed from the PUN NLIN analysis to be consistent between the two parameters.

The optimal SID Lys requirement estimated by the broken-line analysis of GFR (1.03%) was very similar to the one estimated by the analysis of pooled PUN (1.01%). Usually PUN tends to produce lower estimates (Susenbeth, 2006); however, this was not the case for this study. The final SID Lys requirement (1.02%) was the average of the GFR and PUN estimates combining together the performance requirement (GFR) and the physiological one (PUN).

The SID Lys requirement of this study is in close agreement to the NRC (1998) recommendations for the 10 – 20 kg pig (1.01%). It was also similar to previous studies done by Campbell et al. (1988) who recommended a SID Lys level of 1.10% for 8 – 20 kg pigs. Similarly, Martniez and Knabe (1990) found a SID Lys requirement of 1.02% for a crossbred of Yorkshire-Landrace x Duroc-Hampshire weaned at 28-d and fed a 20% CP corn-peanut meal. Later, Mahan et al. (1993) used a corn-SBM with the inclusion of dried whey. The authors found that nursery pig performance reached a maximum at 0.97% of SID Lys. However, more recent studies have shown higher SID Lys levels. Yi et al. (2006) and Kendall et al. (2008) recommended a SID Lys level of 1.32% and 1.30%, respectively. Dean et al. (2007) recommended an even higher level of SID Lys (1.40%). Perhaps one of the reasons for the higher Lys levels observed in those studies could be the modern lean genotypes used in those studies. Hill et al. (2007) suggested an increase in NRC (1998) SID Lys recommendations for modern lean genotypes. Yi et al. (2006) and Kendall et al. (2008) used a PIC commercial line (Dalland x PIC Camborough 22) for their experiments. PIC (2008) suggests SID Lys levels of 1.46% (phase II, 5.5 – 7.3

kg), 1.42% (phase III; 7.3 – 11.4 kg) and 1.30% (11.4 – 22.7 kg) as normal practice to feed their commercial nursery pigs and recommend those feeding levels to achieve maximum performance.

The R^2 is the proportion of the variability of the data that is produced by the model (Steel et al., 1997) and is used to chose the analysis that best fits the data (Robbins et al., 2006). For the case of the GFR analysis, the broken line model yielded a R^2 equal to 0.3586. This value was not provided directly by the SAS output, instead it was calculated by the equation of Robbins et al. (2006). A very similar value was observed for the PUN analysis (0.4070). Robbins et al. (2006) suggested that the best fit broken line analysis should be the one with the higher R^2. We subjected our data to a quadratic-broken-line analysis. The results were the same (including the R^2). Our R^2 values suggest that close to 60% of the variability of our model is due to experimental error (i.e. variability of the individual experimental units). On average, the CV (%) of both parameters was 16.8%, which is a clear indicator of the individual variability of the data.

The SID Lys level obtained from this study (1.02%) confirms the suspicions that the levels of SID Lys used on our previous experiment (~1.30%, Chapter 3) were above the pig requirement, and that perhaps was one of the reasons for the lack of response to Trp supplementation.

4.6 CONCLUSION

Increasing SID Lys level of the diet yielded no differences ($P > 0.10$) in ADG ($P = 0.68$, 0.34 kg/d on average), GFR ($P = 0.38$, 0.60 kg BW gain/kg feed on average) or pooled PUN ($P = 0.23$, 3.85 mmol/l on average).

ADFI was linearly increased (from 545 to 561 g/d) when the SID Lys level was increased from 0.98 to 1.33%. Similarly, GFR was also linearly ($P < 10$) increased (from 0.57 to 0.62 BW gain/kg feed) when the levels of SID Lys increased from 0.89 to 1.33%. Using the broken line analysis the SID Lys requirement was determined at 1.03 ± 0.06 ($P = 0.01$; $R^2 = 0.36$). Similarly PUN decreased (from 5.28 to 3.45 mmol/l) linearly ($P = 0.01$) and quadraticaly ($P = 0.08$). The broken-line analysis suggested a SID Lys requirement of 1.01 ± 0.04% ($P = 0.01$; $R^2 = 0.41$).

Our data suggests a SID Lys level of 1.02% for early-weaned pigs (Yorkshire x Landrace) fed a wheat-barley diet in close agreement to the NRC (1998) recommendations.

CHAPTER 5

MANUSCRIPT THREE

Determining the optimal SID tryptophan-to-lysine ratio for early-weaned pigs fed a wheat-barley based diet.

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5.1. ABSTRACT

Trp requirements are usually expressed as a ratio to Lys. In the last 20 years there have been several studies on the Trp requirements for pigs. However, there are many differences among those results. The objective of this study was to determine the optimal SID TLR of early weaned pigs fed a wheat-barley-based diet. Yorkshire x Landrace pigs (n=72, initial BW: 7.88 ± 0.63 kg) were blocked by BW, group housed (2 pigs/pen; 1 barrow and 1 gilt), and allotted to 6 dietary treatments (6 pen/diet). Supplemental Trp was added to a wheat-barley-Trp-deficient basal diet (0.92% SID Lys; 1.7% SID Trp; 19.99% CP) in order to create graded levels of SID TLR (17.75, 18.18, 19.63, 21.55, 22.73 and 23.61%). Pigs were allowed *ad libitum* access to feed (offered as pellet) and water. Average daily feed intake (ADFI), average daily gain (ADG), gain-to-feed ratio (GFR) and plasma urea N (PUN) were measured weekly and used as response criteria. After 3 wk, 3 barrows from each treatment (initial BW: 15.47 ± 2.30 kg) were selected for a 7-d N balance study (3-d of adaptation followed by 4-d of total collection of faeces and urine). Increasing the SID TLR from 17.75% to 23.61% had no effect on ADFI (P = 0.72, 468 g/d on average), ADG (P = 0.82; 0.29 kg/d on average), GFR (P = 0.81, 0.62 kg BW/kg feed) or PUN (P = 0.46, 4.00 mmol/l on average). Increasing the SID TLR did not cause any differences (P > 0.10) in N retention (22.40% on average) or N excretion (77.60%, on average). No linear or quadratic response was observed (P > 0.10) for any of the parameters evaluated, with the exception of N retention (g/d) which was linearly (P = 0.05) and quadraticaly (P = 0.06) increased from (18.60 to 21.433 g/d) when SID TLR was increased from 17.75 to 23.61%. Analyzing N retention (g/d) with the broken-line analysis, the optimal SID TLR found was outside of the dose

range evaluated for this study ($P = 0.02$). All the other parameters evaluated failed ($P > 0.10$) to yield an estimate. The reason for the lack of effect may be due to the fact that there was an underestimation of the Trp content of the feedstuff used, changing the proposed SID TLR range of the study to higher values (i.e., the targetd values ranged from 13.9 to 21.3%; the analyzed values ranged from 17.8 to 23.6%). Based on the evidence we suggest that the optimal SID TLR for early-weaned pigs fed a wheat-barley diet lies below 17.75%, similar to the NRC (1998) suggestions.

Key Words: lysine, requirements, tryptophan, weaned pigs

5.2. INTRODUCTION

Trp is an essential AA that can be equally first (Sato et al., 1987), second or third limiting AA (Guzik et al., 2002) in diets for weaned piglets. Dietary Trp supply is important because Trp is associated with many biological roles. Among those, Trp is the precursor of serotonin, a neurotransmitter involved in feed intake (Henry et al., 1992) and stress response (Sève et al., 1991). Furthermore, Trp catabolism has also been linked to the immune response (Mellor and Munn, 1999). In addition, adequate Trp supply is key to achieving optimal performance in pigs (Guzik et al., 2002).

Trp requirement for nursery pigs has been researched extensively; however, there is still a large variation in the recommended optimal Trp supplementation with levels ranging from 0.12% (Boomgaardt and Baker, 1973) to 0.23% (Sève et al., 1991). Many factors are thought to be responsible for the differences including CP level, diverse feedstuff used, differences in Trp digestibility (Susenbeth and Lucanus, 2005).

With the application of the ideal protein concept (ARC, 1981) the dietary content of most of the AA is expressed as the ratio to the first limiting AA, generally Lys (Boisen, 2000). On the other hand, in an effort to avoid the difference in digestibility of Trp among feedstuffs, the use of digestible Trp has been proposed (Guzik et al., 2002). Therefore, Trp requirements should be expressed as a ratio to Lys on SID or TID basis (Susenbeth and Lucanus, 2005).

Among the statistical methods to determine AA requirement, the broken line analysis is preferred due to its simplicity (Robbins et al., 2006). Previous research in our group failed to show an effect of Trp in nursery pigs. One of the reasons was that the basal diets may not have been Trp deficient. Hence, the objective of this study was to determine the SID TLR of weaned Yorkshire x Landrace pigs (Iceman Genetics, St. Eustache, MB, Canada) using the broken-line analysis.

5.3. MATERIALS AND METHODS

Timeline

Pigs arrived at the T.K. Cheung Center for Animal Research (TKCCAR) on May 8, 2008. After 4 d of adaptation, the performance trial started on May 12, 2008 and ended on June 02, 2008 (a total of 21 d). The N balance trial started on June 03, 2008 and ended on June 10, 2008 (a total of 7 d).

Animals and Diets

All experimental procedures were reviewed and approved by the University of Manitoba Animal Care Committee (Protocol Nº F05-024/2). Pigs were cared for following the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

For this study, seventy-two Yorkshire x Landrace piglets (36 barrows and 36 gilts) weaned at 17 ± 1 d were obtained from Iceman Genetics (St. Eustache, MB, Canada). After 4 d of adaptation to a commercial starter diet, 4 spare pigs (2 barrows and 2 gilts) were removed based on their BW (the heaviest and lightest from each gender), the average initial BW was 7.88 ± 0.63 kg. Pigs were weighed and blocked on the basis of BW and sex and randomly allotted into groups of two pigs per pen (1 barrow and 1 gilt). Each pen was considered an experimental unit. There were a total of 36 experimental units.

Six experimental units were assigned to one of the six dietary treatments. The N-content ingredients came from the same batch used for the experiment described on section 4.3 (Table 4.1).

The experimental basal diets were mixed at the University of Manitoba feed mill. CGF was included in the formulation to ensure a Trp-deficient basal diet. Two basal diets wheat-barley diets were formulated; 1) a Trp-deficient diet (SID TLR of 17.75%; Diet A) and 2) a Trp-surplus diet (same Trp deficient diet plus supplemental Trp to achieve a SID TLR of 23.61%; Diet F); the other 4 diets were obtained by combining different amounts of the basal diets (Table 5.1). Diets were formulated to differ only in their Trp content. All other nutrients were supplied in amounts meeting or exceeding NRC (1998) recommendations and using Rademacher et al. (2000) SID coefficients, except for Lys which was set to be 10% lower than the level obtained in section 4.4 (Table 5.2). To ensure proper AA mixing, crystalline AA, including crystalline L-Trp (L–tryptophan, Evonik–Degussa, Hanau, Germany), were initially pre-mixed with ~15 kg of ground wheat (a portion of the diet formulation). The wheat-AA premix was included in the feed mill mixing recipe. After mixing all the

Table 5.1. Composition of experimental diets [a] (as-fed basis) used in Experiment 4.

Ingredient	Standardized ileal digestible tryptophan-to-lysine ratio (%)					
	17.75	18.18	19.64	21.56	22.74	23.61
Barley	18.09	18.09	18.09	18.09	18.09	18.09
Soybean meal	1.40	1.40	1.40	1.40	1.40	1.40
Corn gluten feed	37.20	37.19	37.18	37.16	37.14	37.12
Peas	13.27	13.27	13.27	13.27	13.27	13.27
Wheat	11.25	11.25	11.25	11.25	11.25	11.25
Spray dried blood plasma	4.00	4.00	4.00	4.00	4.00	4.00
Dried whey	6.50	6.50	6.50	6.50	6.50	6.50
Vegetable oil	5.00	5.00	5.00	5.00	5.00	5.00
Limestone	1.08	1.08	1.08	1.08	1.08	1.08
Biophos	0.50	0.50	0.50	0.50	0.50	0.50
Vitamin / mineral premix [b]	1.00	1.00	1.00	1.00	1.00	1.00
Biolys	0.25	0.25	0.25	0.26	0.26	0.26
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.20
L-tryptophan	-----	0.01	0.02	0.04	0.05	0.07
L-isoleucine	0.17	0.17	0.17	0.17	0.17	0.17
L-valine	0.09	0.09	0.09	0.09	0.09	0.09

[a] Diet A: Trp deficient (17.75% SID Trp-to-Lys ratio); Diet F: Trp surplus (23.61% SID Trp-to-Lys ratio); Diet B: mixture of 85% Diet A and 15% Diet F (18.18% SID Trp-to-Lys ratio); Diet C: mixture of 70% of Diet A and 30% of Diet F (19.64% SID Trp-to-Lys ratio); Diet D: mixture of 50% of Diet A and 50% of Diet F (21.56% SID Trp-to-Lys ratio); and Diet E: mixture of 30% of Diet A and 70% of Diet F (22.74% SID Trp-to-Lys ratio).

[b] Providing the following per kilogram of diet: vitamin A, 8255 IU; vitamin D3, 1000 IU; vitamin E, 20 IU; vitamin K, 1.5 mg; riboflavin, 7.5 mg; niacin, 30 mg; vitamin B12, 25 µg; pyridoxine, 4.5 mg; biotin, 200 µg; folic acid, 1 mg; thiamin, 4 mg; choline, 781 mg; copper, 10 mg; iodine, 0.6 mg; iron, 130 mg; manganese, 40 mg; selenium, 0.3 mg; zinc, 130 mg.

ingredients, each diet was then pelleted at a temperature of 70ºC and pressure load of 121 lbs for 40 min.

Pigs were group housed in floor pens (length, 1.8 m; x width 1.2 m; x height, 0.9 m height) with plastic-covered expended metal sheet flooring in two controlled temperature rooms (starting at 30ºC in wk one; temperature was reduced 1ºC/wk) with a 12 h light/dark cycle. Each room housed either 24 or 12 pens. Dietary

Table 5.2. Nutrient content of experimental diets [a] (as-fed basis) of Experiment 4.

	Standardized ileal digestible tryptophan-to-lysine ratio (%)					
Item	17.75	18.18	19.64	21.56	22.74	23.61
Dry Matter, g/kg [a]	905.4	904.9	904.1	902.1	901.4	904.8
Ether extract, g/kg [b]	69.7	69.7	69.7	69.7	69.7	69.7
CP, g/kg [a]	199.6	197.1	199.1	199.4	200.0	200.6
CP, % [b]	19.96	19.71	19.10	19.94	20.00	20.06
Crude fiber, g/kg [b]	66.4	66.4	66.3	66.3	66.3	66.3
Digestible Energy, MJ/kg [a]	3.34	3.34	3.34	3.34	3.34	3.33
Net Energy, MJ/kg [b]	2.24	2.24	2.24	2.24	2.24	2.24
Amino acids, g/kg [a]						
Methionine	4.6	4.6	4.6	4.8	4.7	4.7
Cystine	4.0	3.8	3.8	4.0	4.0	4.0
Methionine + Cystine	8.6	8.4	8.4	8.7	8.7	8.7
Lysine	**11.3**	**11.2**	**11.0**	**11.1**	**11.1**	**11.3**
Threonine	7.8	7.6	7.7	7.7	7.8	7.7
Tryptophan	**2.1**	**2.1**	**2.2**	**2.4**	**2.5**	**2.6**
Arginine	11.7	11.6	11.7	11.7	11.8	117.7
Isoleucine	8.2	8.1	8.3	8.4	8.4	8.5
Leucine	15.8	15.4	15.6	15.8	15.8	15.8
Valine	10.6	10.4	10.6	10.8	10.7	10.7
Histidine	5.3	5.2	5.4	5.4	5.4	5.4
Phenylalanine	8.7	8.5	8.6	8.7	8.7	8.7
Glycine	8.3	8.2	8.3	8.3	8.4	8.3
Serine	9.2	8.9	9.0	9.0	9.1	9.0
Proline	13.0	12.8	12.9	12.9	13.5	12.9
Alanine	10.6	10.4	10.5	10.6	10.6	10.6
Aspartic acid	15.1	14.6	14.9	15.0	15.1	14.9
Glutamic acid	32.8	32.5	32.6	32.7	32.9	32.5
Trp : Lys, % (Total AA basis) [a]	18.58	18.75	20.00	21.62	22.52	23.01
SID Trp : SID Lys, % [a]	**17.75**	**18.18**	**19.64**	**21.56**	**22.74**	**23.61**

[a] Diets were analyzed using wet chemistry following the guidelines of Commission Directive (1998, 2000).
[b] Calculated values.

treatments were randomly allocated to each room in such a way that each diet had the same number of experimental units per room (i.e. 4 or 2 pens per diet/room, respectively). Pigs were allowed *ad libitum* access to feed and water through a nipple drinker and a feeder. Diets were offered in a pellet form. The performance trial was conducted over a 3-wk period. Feed disappearance and pig live weight were

monitored weekly and the two parameters used to determine ADG, ADFI and GFR. Weekly blood samples were obtained for the determination of PUN.

Immediately after the performance trial, 3 barrows per treatment were selected (according to their BW, in such a way that they were close to the treatment average; BW = 15.47 ± 2.30 kg) for a N balance trial. Pigs were housed and N balance was conducted as described in section 3.3.1.

Sample preparation and chemical analyses

Samples (~300 g) of diets where sent to Degussa Canada (Burlington, ON, Canada) for CP and AA analysis. Analyses were performed as described in section 3.3.1.

For PUN samples, 10 ml of blood was collected from one pig per pen via jugular vein puncture into sodium-heparin-vacuntainer-tubes (Becton Dickinson, Rutherford, NJ) on days 0, 7, 14 and 21. Blood samples and PUN analysis was done as described in Chapter 3, section 3.3.

Feces and urine samples during the N balance study were obtained and processed as described in Chapter 3, section 3.3.

Calculations

N ingestion, N retention and N excretion were calculated as described on section 3.3.

Statistical analysis

ADFI, ADG and GFR were calculated as described in Chapter 3, section 3.3. Averaged PUN was calculated as the average of the PUN measurements done in wk 2 and 3.

Data residuals and normality were tested as described in section 3.3

Data was analyzed as a completely randomized design using Proc GLM of SAS (SAS Inst., Inc., Cary, NC). When a significant F-value ($P < 0.10$) was indicated by the ANOVA, the Scheffe test was used to compare treatment means. The model used was $y_{ij} = \mu + d_i + e_{ij}$. Where y_{ij} = initial BW, final BW, ADG, ADFI, GFR, initial PUN, averaged PUN, Final PUN, N ingestion, N excretion and N retention of the j'th pen of the i'th SID TLR; μ = population mean; d_i = main effect of the i'th SID TLR; and e_{ij} = residual error of the j'th pen of the i'th SID TLR.

Linear and quadratic responses were evaluated for ADFI, ADG, GFR, averaged PUN, N ingestion, N excretion and N retention as described in section 4.3. For the variables that showed a linear or quadratic response to Trp supplementation, broken-line analysis was performed as described in section 4.3.

5.4. RESULTS

All animals remained healthy during the experimental period except for one piglet from the 22.74% SID TLR treatment that died at the beginning of week 2 as a consequence of the blood sampling.

There were no differences ($P > 0.10$) on the initial BW (7.88 ± 0.23 kg) or the final BW (13.96 ± 0.68 kg) due to SID TLR. ADFI was similar for the six treatments (467.70 ± 27.79 g/d). There were no differences ($P > 0.10$) due to TLR of the diets on ADG (0.29 ± 0.02 kg/d) and GFR (0.62 ± 0.01 kg BW/kg feed; Table 5.3).

There were no differences ($P > 0.10$) due to TLR of the diets on the initial PUN (4.01 ± 0.24 mmol/l), the final PUN (3.94 ± 0.16 mmol/l) or the pooled PUN measure (4.00 ± 0.18 mmol/l; Table 5.4).

Table 5.3.- Performance of early-weaned piglets fed a wheat-barley based diet with six levels of SID TLR.

Item	Standardized ileal digestible tryptophan-to-lysine ratio (%)						SEM	*P* Value	Contrast [b]	
	17.75	18.18	19.64	21.56	22.74	23.61		SID TLR [a]	Linear	Quadratic
Initial BW [c], kg	7.86	7.81	7.83	8.25	7.55	7.99	0.263	0.578	0.841	0.764
Final BW [c], kg	13.76	13.55	14.05	14.72	13.08	14.75	0.825	0.672	0.545	0.922
ADFI [d], g/d	458	441	469	499	438	501	44.755	0.718	0.663	0.931
ADG [e], kg/d	0.282	0.274	0.293	0.304	0.261	0.319	0.032	0.817	0.590	0.991
Gain / Feed [f]	0.616	0.623	0.623	0.603	0.635	0.626	0.016	0.812	0.727	0.768
n [g]	6	6	6	6	6	6				

[a] Effect of the SID Trp-to-Lys ratio of the diet.
[b] Linear or quadratic effect of graded levels of SID Trp-to-Lys ratio of the diet.
[c] Body weight.
[d] Average daily feed intake.
[e] Average daily gain.
[f] Gain-to-feed ratio (kg BW / Kg feed intake).
[g] Observations per treatment (each observation is the average of two pigs; one barrow and one gilt).

Table 5.4.- Plasma urea N and N metabolism in early-weaned pigs fed a wheat-barley based diet with six levels of SID TLR.

Item	Standardized ileal digestible tryptophan-to-lysine ratio (%)						SEM	P Value	Contrast [d]	
	17.75	18.18	19.64	21.56	22.74	23.61		SID TLR [c]	Linear	Quadratic
PUN, mmol/l										
Initial	3.80	4.13	4.21	3.75	3.48	4.31	0.223	0.367	0.661	0.535
Final Measure	4.00	4.13	3.93	3.68	3.85	4.06	0.196	0.631	0.365	0.845
Pooled Measure [e]	3.97	4.36	3.96	3.86	3.87	4.00	0.190	0.460	0.235	0.489
n [f]	6	6	6	6	6	6				
N Balance [g]										
Ingestion, g/d	23.88	21.56	23.28	25.38	24.26	26.59	1.480	0.301	0.111	0.178
Excretion, g/d	5.28	5.35	5.38	5.77	5.50	5.16	0.733	0.994	0.861	0.821
Excretion, %	22.02	24.85	23.28	22.58	22.63	19.04	2.291	0.636	0.413	0.226
Retention, g/d	18.60 [ab]	16.21 [b]	17.90 [ab]	19.61 [ab]	18.76 [ab]	21.43 [a]	1.096	0.088	0.048	0.059
Retention, %	77.98	75.15	76.72	77.42	77.37	80.96	2.291	0.636	0.413	0.226
n [f]	3	3	3	3	3	3				

[a,b] Means with different superscript are statistically different ($P < 0.10$)
[c] Effect of the SID Trp-to-Lys ratio level of the diet.
[d] Linear or quadratic effect of graded levels of SID Trp-to-Lys of the diet.
[e] Means are the average of measurements done on day 14 and 21.
[f] Observations per treatment.
[g] As feed basis

There were no linear or quadratic effects ($P > 0.10$) due to TLR of dietary treatments on initial BW, final BW, ADG, ADFI, GFR, initial PUN, final PUN and pooled PUN (Table 5.3 and 5.4)

No differences were found ($P > 0.10$) in N intake (24.76 ± 1.73 g/d) or N excretion (5.41 ± 0.21 g/d or 22.40 ± 1.91 %) due to the TLR of the treatments (Table 5.4). The pigs fed the 23.61% SID TLR diet retained more ($P = 0.09$) N (g/d) than those fed the 18.18% SID TLR diet (21.43 ± 1.39 vs. 16.21 ± 0.96 g/d). However when this variable was expressed as a percentage of N intake, no differences ($P > 0.10$) were observed among the treatments (77.60 ± 1.91%).

There was a linear ($P = 0.05$) and quadratic ($P = 0.06$) increase in N retention (from 18.60 to 21.433 g/d) when the SID TLR was increased (Figure 5.1). There were no linear or quadratic effects ($P > 0.10$) on any of the others parameters measured in the N balance study.

Subjecting N retention (g/d) to the broken-line analysis, the optimal SID TLR found was outside of the dose range evaluated for this study ($P = 0.02$; Figure 5.2). All the other parameters evaluated failed ($P > 0.10$) to yield an estimate.

5.5. DISCUSSION

The purpose of this study was to determine the optimal SID TLR of early-weaned pigs (Yorkshire x Landrace) fed a wheat-barley diet. The SID Lys level selected (0.92%) was set to be 10% lower than the result obtained on a previous experiment (Chapter 4). Setting the Lys requirement to this level was done to maximize the utilization of Lys (first limiting AA) and Trp (second limiting AA for

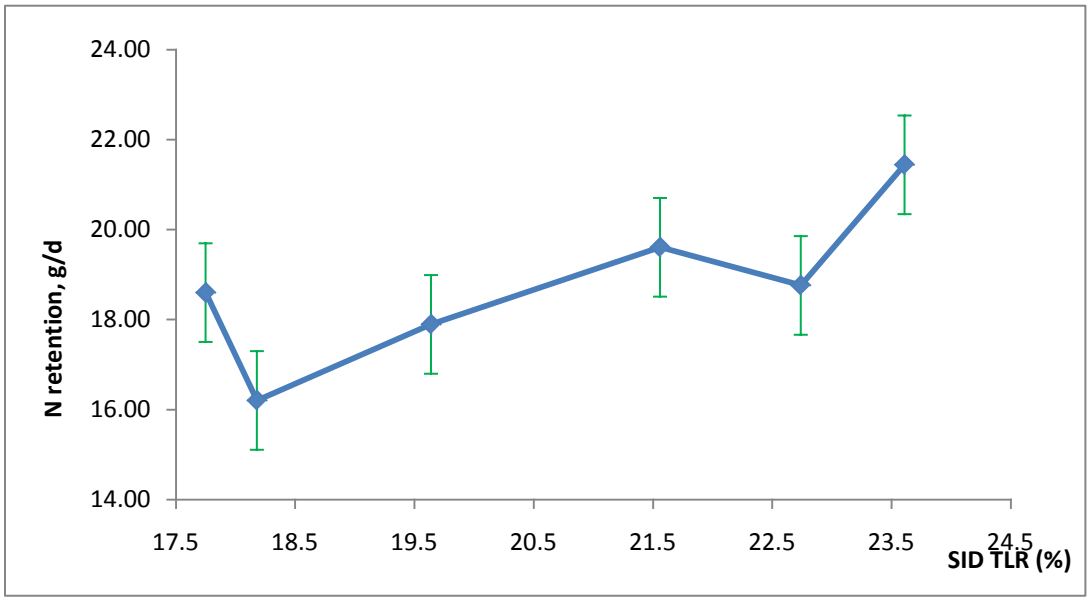

Figure 5.1. N retention (g/d) response of nursery pigs fed a wheat-barley diet with graded levels of SID Trp-to-Lys ratio (Linear: *P* = 0.05; Quadratic: *P* = 0.06).

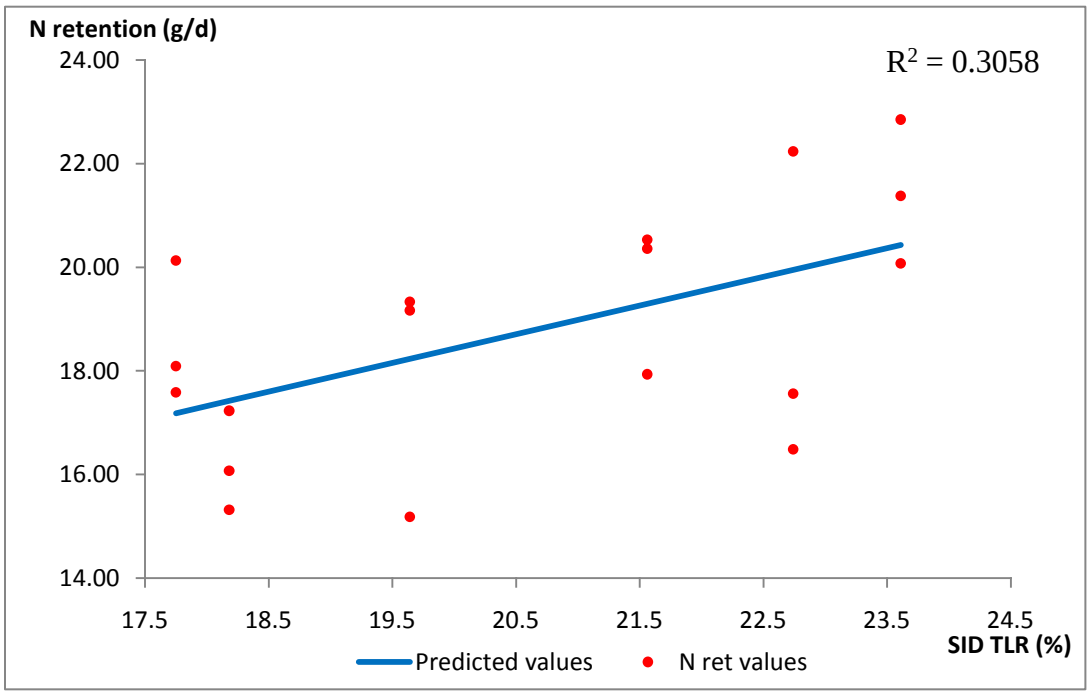

Figure 5.2. Determination of SID Trp-to-Lys requirement using the broken-line analysis and N retention (g/d) as a response criteria.

Equation line : Pred N ret = L + U * (Z1). Where: Pred N ret = predicted value of N ret; L = Asymptote = 22.587 ± 1.160; U = Slope = -0.554 ± 0.209; Z1= (SID TLR < Requirement) * (Requirement – SID TLR). Final SID TLR was outside of the evaluated range (*P* = 0.02)

this study) by the piglets (Van Cauwenberghe and Relandeau, 2000). CGF was added to the diets to ensure a Trp-deficient basal diet.

Diets were pelletized to reduce the feed waste (it is easier to adjust the feeders to avoid waste using pellets instead of mash) and to reduce the work associated with weighing of feed refusal.

CP levels were in close agreement with the proposed values (19.80 ± 0.36% vs. 19.25%). Similarly the analyzed SID Lys of the diets (0.93 ± 0.01) was in close agreement to the proposed one (0.92%). However there were differences between the proposed SID Trp levels (0.13, 0.14, 0.15, 0.16, 0.18 and 0.20%) and the analyzed values (0.17, 0.17, 0.18, 0.20, 0.21 and 0.22%). As a consequence of these differences, the proposed SID TLR were shifted to higher values (13.91 vs. 17.75%, 15.02 vs. 18.18%, 16.12 vs. 19.64%, 17.59 vs. 21.56%, 19.06 vs. 22.74%, and 21.26 vs. 23.61%). Overall the analyzed values of the rest of the essential AA were higher than the proposed ones with the exception of Met (which was a little bit lower) and Cys (which was in close agreement with the proposed values).

Taking into account that only 2 basal diets were mixed (a Trp-deficient and a Trp-surplus), individual weighing errors were not the source of the differences for the other experimental diets. These differences can be due to laboratory underestimation of the samples. The root cause of these differences between the calculated and analyzed values was not due to the mixing process. This was confirmed with the levels of SID Lys and CP that were in close agreement with the proposed ones. Perhaps one of the reasons for these variations, is that during the AA analysis of the N-containing ingredients (Table 4.2) there was an underestimation of Trp. Feedstuff samples were sent to the Experiment Station

Chemical Laboratories (University of Missouri, Missouri, CO) for re-analysis of AA and CP. The new set of analysis confirmed that Trp content of peas (0.18 vs. 0.20%), wheat (0.15 vs. 0.18%), SBM (0.64 vs. 0.71%), dried whey (0.19 vs. 0.23%) and SDBP (1.40 vs. 1.56%) were underestimated on our original analysis. Similarly, the new analysis of Lys and the other essential AA showed the same tendency. For CGF both analyses were in close agreement, especially for Trp that yielded the same value on both analyses (0.18%).

Peas, wheat and SBM were originally analyzed using NIR spectroscopy (Table 4.1). The new analyses were performed using wet chemistry and following the guidelines of the AOAC (2006) Official Methods 988.15 E (a) for Trp and 982.30 E (a, b) for the other AA. Wu et al. (2002) failed to quantify the amount of Trp on rice samples using NIR spectroscopy due to its low concentration, suggesting the fluorometric method to better determine Trp content in cereals. Fontaine et al. (2002, 2004) reviewed the efficacy of NIR spectroscopy on predicting AA content of feedstuff. Overall the Trp predictions had higher coefficient of variation (CV) and coefficient of determination (R^2) compared to the other AA. CGF was analyzed by wet chemistry on both rounds of analyses, and no differences were found. However, SDBP was also analyzed by wet chemistry on both rounds of analyses with similar results on AA content except for Trp that was lower (1.40 vs. 1.56%) on the first set of analyses. It is not unusual to find variations on AA evaluation among laboratories (Cromwell et al., 1999) and those differences are greater when analyzing Trp (Sato et al., 1984). The authors found that the fat content of the feed ingredient can lead to an underestimation of Trp values.

Since there was no effect of the SID TLR on the initial BW, there was no need to perform the analysis of variance using the initial BW as a cofactor. All the parameters evaluated (i.e. BW, ADG, ADFI, GFR, PUN, N ingestion, N excretion and N retention) were checked to ensure the assumptions of the analysis of variance were met (Steel et al., 1997). Normality of the residuals was evaluated using the Shapiro-Wilk test of SAS (SAS Inst., Inc., Cary, NC).

ADFI was similar to earlier studies (530 g/d, Burgoon et al., 1992; 553 g/d, Han et al., 1993; 559 ± 54 g/d, Eder et al., 2001) and was also similar to the NRC (1998) reported values for feed intake (Table 5.1). Guzik et al. (2002) evaluated the effect of graded SID TLR (from 12.87 to 25.25%) in 144 piglets (initial BW of 10.3 kg) fed a corn-pea diet (1.01% SID Lys). Their experiment yielded higher ADFI (717 g/d), similar ADG (0.38 kg/d) and lower GFR (0.53 kg BW gain/kg feed) than our study. This higher ADFI could be a consequence of the higher initial BW of that trial.

As shown in Table 5.3 and 5.4, with the exception of N retention (g/d), there were no linear or quadratic effects ($P > 0.10$) on all the parameters evaluated when the levels of SID TLR were increased from 17.75 to 23.61%. These results are in contradiction with previous studies. Burgoon et al. (1992) evaluated the effect of graded levels of Trp on nursery pigs (initial BW = 6.2 kg) fed a corn-SBM diet (22% CP and 1.21% SID Lys). The authors found linear and quadratic increases ($P < 0.01$) when the SID TLR was increased from 10.07 to 17.03%. Similarly, Eder et al. (2001) evaluated 4 graded levels of Trp (13.82, 16.26, 18.70 and 21.14% SID TLR) in a wheat-barley-pea diet (15.6% CP and 1.08% SID Lys) fed to nursery pigs (initial BW ~ 8 kg). The authors found an increase in ADFI (from 538 ± 95 to 637

± 59 g/d) and ADG (from 360 ± 73 to 445 ± 42 g/d) when the SID TLR was increased from 13.82 to 16.26%; however, there were no statistical differences (P > 0.05) between the 16.26% and the higher SID TLR. Guzik et al. (2002) evaluated 6 graded levels of SID TLR (9.61, 11.83, 14.05, 16.27, 18.49 and 20.71%) in a corn-pea diet fed to phase II nursery pigs (from 6.3 to 10.2 kg). They also found a linear and quadratic increase (P < 0.05) on ADG, ADFI and GFR when Trp levels were increased in the diet. Also, similar results were obtained by Eder et al. (2003) with grower-finisher pigs.

Two hypotheses can be argued to explain the lack of effect of Trp supplementation in our study. The first one could be that the pigs were overfed Lys, and that the Lys excess caused the lack of response of pigs towards Trp supplementation (as discussed in section 3.5). This is highly unlikely because Lys requirement of the exact same breed was predetermined in a previous experiment (Chapter 4). Furthermore pigs came from the same supplier. Hence, SID Lys was set at 0.92%, which is 90% of the optimal SID Lys level found in the previous experiment (1.02%, Section 4.6).

The second one, which is the more plausible one, is that our basal Trp deficient diet was in fact not-deficient. Susenbeth (2006) mentioned that in order to find a clear response to graded levels of an AA, the dose range should cover levels of deficiency, adequacy and excess. Recent studies suggested that the SID TLR was between 15 and 17% (Guzik et al., 2002; Susenbeth, 2006). Hence in order to cover levels from deficiency to surplus, the SID TLR were targeted to range from 13.91 to 21.26% (i.e. 2 or 3 levels below the suspected optimal, and 4 or 3 levels above). However, due to the underestimation of Trp content of the feed ingredients,

the analyzed SID TLR ranged from 17.75 to 23.61%, the lower analyzed level theoretically was already above the optimal requirement.

This second hypothesis is supported by the findings of the previously mentioned studies (Burgoon et al., 1992; Eder et a., 2001; Guzik et al., 2002). For example, Burgoon et al. (1992) suggested an optimal total Trp supplementation of 0.19% (equivalent to 13.77% SID TLR). The authors tested 3 levels below (from 10.07 to 12.83%) and 3 levels over (from 14.64 to 17.03%) their recommended optimal. A similar tendency was observed in the study by Guzik et al. (2002) where the optimal Trp requirement was estimated to be 0.20% digestible Trp (equivalent to 17% SID TLR). These authors tested 4 levels below their estimated and 2 levels over. A clearer picture is presented in the study of Eder et al. (2001). Their estimates yielded an optimal dietary Trp concentration of 2.09 g/kg (equivalent to 16.99% SID TLR). In this case there was one level below the optimal recommendation (13.82% SID TLR), one very close to the recommendation (16.26%) and two levels over (18.70 and 21.74%). The authors observed an increase in pig performance when Trp supplementation shifted from suboptimal to optimal, and there was no increase when the Trp supplementation reached the surplus levels. That confirms the notion that once the Trp requirement is met, there will be no effect of added Trp above that level (Susenbeth, 2006). Furthermore, some authors (Borg et al., 1987; Seve et al., 1991; Henry et al., 1996) had demonstrated a marked decrease on feed intake when pigs were fed inadequate levels of dietary Trp due to the role of this essential AA as a precursor of serotonin. Deficient dietary Trp levels can reduce the uptake of Trp by the brain and reducing the synthesis of serotonin (Sève et al., 1991). In our study no differences were

found ($P > 0.10$) among the 6 dietary Trp levels tested, confirming the idea that the lowest level of SID TLR was not deficient in Trp supplementation.

The lack of response in terms of Trp supplementation (Table 5.4), also supports the idea that the Trp level was not deficient in the experimental diets. Brown and Cline (1974) showed that when the requirement of a deficient AA is met (Trp in this case) urea excretion is minimized due to a better N utilization (Coma et al., 1995). PUN tends to decrease when the requirement of the tested AA shifts from deficiency to adequacy (Coma et al., 1995; Guzik et al., 2002, 2005; Susenbeth, 2006). The lack of linear or quadratic decrease ($P > 0.10$) in this study confirms that there was no shift because there was no dietary Trp deficiency to start with. Previous studies also found a decrease in PUN levels when Trp supplementation was increased from suboptimal to optimal. Leibholz (1981) found a decrease on PUN in 28 d old piglets when dietary Trp supplementation was increased. Similarly Lewis et al. (1977) also found a decrease in PUN when the Trp dose was increased from deficiency to adequacy. Also, Zimmerman (1975) found linear and quadratic decrease in PUN when the levels of Trp were increased from 0.10 (deficiency) to 0.23% (surplus).

In our study, N retention (g/d) was increased linearly and quadraticaly with the addition of graded levels of Trp. Similar results were obtained by Eder et al. (2003). The increase in N retention (g/d) in our study was not observed when N retention was expressed as a percentage of intake. Suggesting that the variation was due to differences in N intake among barrows.

The evaluation of dose-response studies has been done with regression of analytical models of the linear or non-linear analysis (Robbins et al., 2006). The

advantage of non-linear models is that they describe better the physiological behaviour and with the new NLIN procedures of SAS can easily be calculated (Robbins et al., 2006). N retention (g/d) was subjected to the broken-line analysis (this was the only variable of our study that showed a linear or quadratic effect towards Trp supplementation). The analysis did not yield an optimal SID TLR within the experimental range. In dose response studies the optimal requirement is defined "as the maximum of the intake were the plateau of the dose-response relationship is reached" (Susenbeth, 2006). The non-linear analysis chooses the optimal requirement when the error sum of squares is minimal (Robbins et al., 2006). In our study the maximum response and the minimal error sum of squares were outside of the tested range (the optimal SID TLR yielded by the analysis was 27.5%, $P = 0.02$). All of the other variables failed to yield ($P > 0.10$) an optimal requirement. Similar to our results, Susenbeth and Lucanus (2005) failed to find any effect on daily gain due to dietary Trp supplementation. The authors tested 6 levels of SID TLR (from 17.5 to 24.5%) on a wheat-barley diet (20.9% CP; 1.25% SID Lys DM basis) fed to nursery pigs (between 15 – 25 kg BW). They concluded that the optimal TLR was not determined due to the lack of a Trp-deficient basal diet, suggesting that the optimal TLR was below 17.5%.

Some authors have evaluated the TLR in wheat-barley diets (Jansman et al., 2000; Guzik et al., 2005). Jansman et al. (2000) study yielded an optimal SID TLR of 20.9%. They evaluated 3 SID TLR (14.8, 18.0 and 20.9%) in nursery pigs (between 9 to 28 kg BW), and their optimal TLR was derived from the maximum ADG, which increased linearly with the increase in dietary Trp supplementation. More recently, Guzik et al. (2005) tested 3 TLR (14.5, 17.0 and 19.5%) in a pea-

wheat-barley diet fed to nursery pigs. They found a linear increase in ADG when SID TLR was increased, suggesting an optimal SID TLR of 19.5%. The NRC (1998) recommends an optimal SID TLR of 18% for nursery pigs. More recently (Susenbeth and Lucanus, 2005) suggested an optimal SID TLR between 17 and 18%.

Based on the evidence, we suspected that the optimal TLR was below our lowest analyzed ratio, suggesting that the lack of response of Trp supplementation of the present study is due to a lack of a Trp-deficient basal diet.

5.6. CONCLUSION

Increasing SID TLR levels of the diet did not affect ADFI (P = 0.72; 467.70 g/d on average), ADG (P = 0.82; 0.29 kg/d on average), GFR (P = 0.81; 0.62 kg BW/kg feed on average) or pooled PUN (P = 0.46; 4.00 mmol/l on average).

No effects of Trp supplementation were also observed on N intake (P = 0.30; 24.76 g/d on average), N excretion (P = 0.64; 22.40% on average) or N retention (P = 0.64; 77.60 % on average).

No linear or quadratic responses were observed (P > 0.10) for any of the parameters evaluated, with the exception of N retention (g/d) which was linearly (P = 0.05) and quadraticaly (P = 0.06) increased from (18.60 to 21.43 g/d) when SID TLR was increased from 17.75 to 23.61%.

Analyzing N retention (g/d) with the broken-line analysis, the optimal SID TLR found was outside of the dose range evaluated for this study (P = 0.02). All the other parameters evaluated failed (P > 0.10) to yield an estimate.

Based on the evidence we suggest that the optimal SID TLR for early-weaned pigs fed a wheat-barley diet lies below 17.75%, similar to the NRC (1998) recommendations.

CHAPTER 6

GENERAL DISCUSSION

Trp is an essential AA that can not be synthesised by mammals which depend on daily intakes of dietary Trp to meet their requirements (Comai et al., 2005). Trp is involved in many biological functions. In addition to being used by the body for protein synthesis, Trp is the precursor of serotonin (Allegri et al., 2003) and NAD^+ (Schröcksnadel et al., 2006). Furthermore, Trp supplementation has been related to an increase in feed consumption (Eder et al., 2001), reduction of stress levels (Sève et al., 1991; Koopmans et al., 2005) and with the immune response (Moffett and Namboodiri, 2003). Trp is catabolised in the body through two principal metabolic routes. The major one, in terms of quantity, is the kynurenine pathway where 90% of the total Trp is catabolised (Comai et al., 2005). Two enzymes regulate this metabolic route: a hepatic enzyme (TDO; Allegri et al., 2004) and an extrahepatic (IDO; Widner et al., 2000). The main product of this pathway is kynurenine. The second in importance is the serotonin pathway. Close to 1% of the total Trp ingested is converted to serotonin in the brain (Le Floc'h and Sève, 2007). Serotonin is also the precursor of melatonin, a neurotransmitter involved in the regulation of the circadian rhythm (Reiter, 2003).

In an effort to reduce feeding cost and pollution in swine facilities, low CP diets supplemented with AA are being used more frequently (Kerr et al., 2003; Shriver et al., 2003; Khendal et al., 2007). However, feeding inadequate levels of Trp can lead to a decrease in pig performance (Henry et al., 1992). The optimal Trp

supplementation for pigs have been studied extensively, however, there are clear variations in the recommended dietary levels of Trp supplementation (Burgoon et al., 1992). Those variations are related to genetics, CP level and type of feedstuff used, among other factors (Guzik et al., 2002). With the concept of the ideal protein (ARC, 1981), AA ratios are set relative to the first limiting AA usually Lys (Boisen, 2000). For weaned piglets, the literature is diverse on the optimal TLR, which ranges from 14% (Burgoon et al., 1992) to ~21% (Jasman and Van Diepen, 2005). The objective of this thesis was to determine the effect of CP and diet type on the optimal TLR of weaned pigs.

In Chapter 3, the effect of dietary composition and CP level over the optimal TLR was evaluated. Two diet types were tested: a USA (US) type of diet (Corn-SBM) and a Western Canadian (WC) type of diet (Corn-Wheat-Barley-Peas-SBM), and two levels of SID TLR were assessed (15 and 19%). Pigs fed the WC diet had lower ($P < 0.10$) ADG, GFR and as a consequence lower final BW than those fed the US diet. Perhaps one of the reasons for this difference between diet types was the presence of ANF on the WC diet. Le Guen et al. (1995) tested whole-pea-diets (~25% of raw pea) vs. isolated-pea-protein-diets (the latter diet had on average 60% less of trypsin inhibitor and lectins activities compared to the whole-pea-diet; two well known ANF). They found a lower ADG on the pigs fed the whole pea diet vs. the one fed the isolate-diet. Overall there were no effects of the interaction of diet type and TLR. These results differed from those of a previous study (Jansman and Van Diepen, 2005) that suggested an increase in the TLR for pigs fed corn-SBM diets as opposed to wheat-barley diets. A more recent study (Jansman et al., 2010), also found an increase in performance of pigs fed a wheat-barley diet and high

levels of TLR; however, they concluded that TLR is not dependant of dietary composition. In the present experiment no effects ($P > 0.10$) of the TLR were found on most of the parameters evaluated (i.e., ADFI, ADG, GFR, averaged PUN, N ingestion, N excretion and N retention). These results were in contradiction with previous studies (Burgoon et al., 1992; Henry et al; 1992, 1996; Eder et al., 2001). The second experiment utilized a corn-SBM diet to evaluate two levels of CP (17 and 20%) and two levels of SID TLR (16 and 20%). The corn-SBM was chosen over the WC diet due to its better ADG, GFR and final BW obtained in Experiment 1. Pigs fed the higher CP diet had higher ($P < 0.10$) ADG, GFR and final BW than those fed the lower CP diet. Similar to Experiment 1, TLR was not affected by the CP level of the diet. This was in contradiction with results from Jansman et al. (2010). They found an increase in ADG, ADFI and GFR in pigs when the Trp-to-BCAA was highest. BCAA and Trp compete for transport across the blood-brain-barrier, hence a higher Trp-to-BCAA ratio will increase the availability of Trp for serotonin synthesis and improve feed intake (Henry et al., 1996) and performance.

Both experiments showed a lack of an effect of the TLR and the interaction with diet type (Experiment 1) or CP level (Experiment 2) on the parameters evaluated. Perhaps one of the reasons behind this lack of effect of Trp supplementation could be the level of Lys in the experimental diets;,which possibly was above the pigs' requirement. On average, for both experiments, SID Lys was set to 1.31% following Degussa recommendations for weanlings (1.35% SID Lys). Van Cauwenberghe and Relandeau, (2000) said that an excess of Lys (above the requirement) can underestimate the optimal TLR due to the fact that CP and other non-essential AA will become limiting (Susenbeth, 2006). For dose response trials,

and to find the true effect of the AA being tested, the levels of Lys should be set between 90 or 95% of the requirement (Barea et al., 2009). Regarding the optimal SID Lys supplementation of weaned piglets, there is vast range of recommendations from 1.19% (NRC, 1998) to values higher than 1.30% (Yi et al., 2006; Khendall et al., 2008). The second reason for the lack of effect of Trp supplementation in the present studies may have been that TLR used was above requirement. In Experiment 2 the analyzed lower SID TLR was close to 18%. It has been pointed out that in order to observe an effect of Trp supplementation, the doses tested should cover the range from deficiency, adequacy and surplus (Susenbeth, 2006). In this case both levels were theoretically above the Trp requirement. Susenbeth and Lucanus (2005) suggested that the optimal TLR should be close to 17%. In view of the results of these two experiments, we decided to add two extra experiments to the project. The objective of the first one was to determine the optimal SID Lys requirement of early weaned pigs fed a wheat-barley diet. With that information at hand, the objective of the subsequent experiment was to find out the optimal SID TLR of early weaned pigs fed a wheat-barley diet.

Chapter 4 was devoted to determine the SID Lys requirement of early weaned pigs fed a wheat-barley diet (typical swine diet representative of Western Canada). There was a linear increase ($P < 0.10$) in ADFI and GFR when the SID Lys level was increased from 0.89 to 1.33%. Similarly PUN also was linearly reduced ($P < 0.10$) when the SID Lys levels were increased. Since urea excretion is minimized when the requirement of the limiting AA (Lys in this experiment) is met (Brown and Cline, 1974), PUN is considered a good measure in the determination of AA requirements (Coma et al., 1995; Guzik et al., 2002, 2005; Susenbeth, 2006). The

increase in GFR and the decrease in PUN will suggest a better N utilization as a consequence of reaching the Lys requirement (Coma et al., 1995). The broken-line analysis is an easy and accurate method to determine the optimal AA requirement (Robbins et al., 2006). Using the broken-line analysis (with the PROC NLIN of SAS), GFR yielded SID Lys requirement estimate of 1.03%, and PUN yielded a very similar one (1.01%). The final SID Lys requirement (1.02%) was obtained by averaging GFR and PUN estimates; combining the estimates obtained using the performance parameter (GFR) and the physiological one (PUN). The R^2 obtained for both estimates (R^2 ~ 0.40) suggested that there was high variability among experimental units. Our estimate was similar to previous research (Martniez and Knabe, 1990; Mahan et al., 1993 NRC, 1998). However, more recent studies yielded higher SID Lys requirement estimates (Yi et al., 2006; Dean et al., 2007; Kendall et al., 2008). Perhaps one of the reasons for these higher estimates is that those studies used higher lean deposition breeds, which required higher levels or Lys to achieve their superior growing potential (Hill et al., 2007).

With the information obtained in Chapter 4, the objective of Chapter 5 was to determine the optimal SID TLR of early weaned pigs (these pigs were from the same breed and supplier than those used on Chapter 4) fed a wheat-barley diet. Following Cauwenberghe and Relandeau, (2000) recommendations for dose studies, SID Lys level was set at 0.92% (i.e. ~ 10% lower than the estimate determined in Chapter 4). A very similar diet composition to the one used in Chapter 4 was used, even with the ingredients coming from the same batch. The only differences were that there were no graded levels of SID Lys, instead there were graded levels of SID Trp. The targeted and analyzed dietary values were

different especially for Trp (i.e. analyzed Trp values were higher than targeted values). Even though there were still graded levels of SID TLR (from 17.75 to 23.61%), this ratios were higher than the proposed ones (from 13.91 vs. 21.26%). The reason for this variance was an underestimation of the Trp concentration of peas (0.18 vs. 0.20%), wheat (0.15 vs. 0.18%), SBM (0.64 vs. 0.71%), dried whey (0.19 vs. 0.23%) and SDBP (1.40 vs. 1.56%). Those samples were initially analyzed with NIR spectroscopy. Fontaine et al. (2002, 2004) found that Trp predictions using the NIR spectroscopy had higher coefficient of variability and R^2 than the predictions for the other essential AA. It is normal to find differences between laboratories on AA analyses; however, those differences are usually greater for Trp (Sato et al., 1984).

There were no linear or quadratic effects ($P > 0.10$) on any of the parameters evaluated when the SID TLR was increased from 17.75 to 23.61% (the only exception was N retention, g/d). These results were in disagreement with previous studies. Burgoon et al. (1992) found a linear and a quadratic increase on performance when SID TLR was raised from 10.07 to 17.03%. A few years ago, Eder et al. (2003) also found an increase on ADFI and ADG when SID TLR was increased from 13.82 to 16.26%. Another study (Guzik et al., 2002) also found linear and quadratic increases on performance when SID TLR was increased from 9.61% to 20.71%. Since N retention (g/d) was the only variable of our study that showed a linear or quadratic effect towards variation of TLR, it was subjected to the broken-line analysis; however, the analysis did not yield an optimal SID TLR within the experimental range tested (the optimal SID TLR yielded by the analysis was 27.5%, $P = 0.02$). In a previous study done by Susenbeth and Lucanus (2005),

the range of TLR was set between 17.5 to 24.5%. They could not found an optimal TLR, and suggested that the optimal was below their lowest TLR. In lieu of that, we speculate that our lowest SID TLR (17.75%) was already above the pig requirement. In order to obtain a clear response to graded levels of a particular AA, the dose range should cover at least the levels of deficiency, adequacy and surplus (Susenbeth, 2006). The original targeted SID TLR were intended to cover a range from 13.91 to 21.26%, i.e. we planned 2 or 3 levels below the optimal and 4 or 3 levels above but due to the underestimation of the Trp concentration in feed ingredients, our lowest interval was shifted to 17.75%. Recent studies suggested that the SID TLR was between 15 and 17% (Guzik et al., 2002; Susenbeth, 2006). Apparently our lowest value was already above the optimal TLR.

It can, therefore, be concluded that there was no effect of diet type or CP level in the SID TLR. Similarly there was also a lack of the interaction between those two factors with SID TLR on the parameters evaluated mainly due to an excess of Lys (1.31% on average) of the experimental diets, which covered the effect of TLR. The optimal SID Lys requirement estimated with the broken-line analysis of early-weaned pigs fed a wheat-barley diet was 1.02%, in close agreement with the NRC (1998) recommendations. Since no effect of graded levels of SID TLR (from 17.75 to 23.61%) was observed on early weaned pigs fed a wheat-barley diet and taking into account that the broken-line analysis failed to yield an estimate within that range, we suspect that the optimal TLR is below 17.75%, in close agreement with previous work (Guzik et al., 2002; Susenbeth, 2006).

CHAPTER 7

CONCLUSIONS

Based on the results of this thesis, it can be concluded that:

1. There was a lack of effect of Trp supplementation and/or the interaction with CP and diet type on the performance and N balance parameters evaluated. The evidence suggests that the diets had a surplus of Lys hence the lack of effect of the variation of TLR.
2. Using GFR and PUN as response criteria and subjecting those parameters to the broken-line analysis, the optimal SID Lys requirement of early-weaned pigs fed a wheat-barley diet is 1.02%, in close agreement with the NRC (1998) recommendations.
3. Broken-line analysis failed to yield an optimal SID TLR within the dose range evaluated (17.75 to 23.61%); however, the evidence suggest that the optimal SID TLR of early weaned pigs fed a wheat-barley diet is below 17.75%.

Recommendations:

1. In the future, dose response studies are advisable to obtain a predetermination of the Lys requirement of the animals. Then it will be necessary to set the levels of Lys at 90% of those values, to obtain a clear response of the tested AA.
2. To determine TLR it is advisable to use as much levels as possible, having more graded levels will allow applying more complex mathematical models, which can yield better estimates.

3. On dose response studies it is imperative to ensure a basal diet which is deficient in the tested AA otherwise a clear response will not be obtained from the study.
4. Finally, more research is needed to determine the optimal TLR of weaned pigs and to evaluate the true effect of CP and diet type over the Trp requirement.

CHAPTER 8

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