Feed intake, growth and carcass composition of weaned piglets receiving varying levels of valine and leucine in their diets

by

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DECLARATION

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

SIGNATURE: ……

DATE: ……

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List of Abbreviations

AA	amino acid
ADG	average daily gain
ADFI	average daily feed intake
ADPG	average daily protein gain
ADVI	average daily valine intake
AID	apparent ileal digestible
ANF	anti-nutritional factor
BCAA	branched-chain amino acid
BCKA	branched-chain α-keto acid
BW	body weight
CP	crude protein
DDGS	dried distiller's grains and solubles
DE	digestible energy
DM	dry matter
EAA	essential amino acid
FCE	feed conversion efficiency
F/G	feed:gain
FI	feed intake
GE	gross energy
GIT	gastrointestinal tract
Lys	lysine
ME	metabolisable energy
MGM	maize gluten meal
MJ	mega joule
NDF	neutral detergent fibre
NEAA	nonessential amino acid
PD_{max}	maximum potential protein deposition per unit time
RMS	residual mean square
SID	standardised ileal digestible
Val	valine

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ABSTRACT

The objective of this experiment was to determine the response of weaned piglets to dietary valine and to quantify antagonistic effects of excess dietary leucine on this response. 72 Large White x Landrace entire male piglets at 13.46 ± 1.18 (mean ± SD) kg were assigned to one of six dietary valine treatments [11.9 (T1), 10.1 (T2), 8.3 (T3), 6.6 (T4), 4.8 (T5) g/kg and T5 + supplemented valine (T6)] and one of three leucine treatments [standardised ileal digestible (SID) valine: leucine ratio of 0.52, 0.27 and 0.16]. Animals received feed *ad libitum* for a period of 18 days. A dilution technique was used to measure responses in feed intake, growth rate and carcass composition to a series of diets containing different valine and leucine concentrations. The summit diets were formulated to contain all amino acids (AAs) other than valine at 1.3 times the requirement and valine at 1.1 times the requirement. A dilution diet, devoid of protein but similar in all other respects, was used to blend the three summit diets to give three series of decreasing valine concentrations. Summit diet 1 was not supplemented with leucine. Summit diet 2 and summit diet 3 were supplemented with 20 g and 40 g leucine/ kg feed respectively to create two diets of moderately and severely imbalanced valine to leucine ratios. Responses in feed intake (FI) and growth rates to varying levels of valine and leucine were observed, but only a response in carcass composition was seen when decreasing dietary valine concentrations were offered to pigs. At normal leucine levels, FI increased as dietary valine concentration decreased. Initially there was an increase in average daily gain (ADG), but as dietary valine concentration dropped, feed conversion efficiency (FCE) decreased and a higher FI did not result in higher ADG. Consequently, body lipid content increased as dietary valine concentration decreased. At moderately excess dietary leucine levels, FI increased initially as dietary valine concentration decreased and dropped sharply as valine content decreased below 8.3 g/kg. ADG initially increased as FI increased, but ADG dropped when FI dropped. FCE dropped and body lipid content increased as dietary valine concentration decreased. At severely excess dietary leucine levels, FI, ADG and FCE dropped and body lipid content increased as dietary valine concentration decreased. In conclusion, leucine did interact with valine and increasing the leucine to valine ratio resulted in poorer growth, but only at low valine concentrations. The depression of FI was not obvious at high valine levels, but the diet with the lowest valine content, which was most severely imbalanced, caused FI and growth to be severely depressed. However, valine supplementation to such an imbalanced diet was found to reverse such a depression of feed intake and growth. This confirms that excess leucine levels, which could often occur in commercial feeds containing

dried distillers grains with solubles (DDGS) and maize gluten, may cause an imbalanced AA pattern which affects growth and feed intake, especially when valine is given in low amounts. When the Reading Model was fitted to the response data for each leucine series, the amount of valine required per kg of body weight for maintenance was found to be 29.4 mg and 67.3 mg per g of protein gain. These coefficients were unaffected by the amount of leucine in the feed. The efficiency of valine utilisation for protein growth was determined to be 73 % and that an intake of around 9 g valine per day will yield maximal protein growth in pigs of the genotype used, and over the period of growth used in this trial.

CHAPTER 1

INTRODUCTION

Before production of synthetic amino acids, protein was included in diets at rates far above animal requirements as a safety margin against amino acid (AA) deficiencies. However, this was not always economically viable due to the high cost of excess protein inclusion and energy costly processes relating to the breakdown of oversupplied AAs by the animal and their excretion in the form of urea. An additional consequence thereof was increased nitrogen pollution of the environment. Economic and environmental pressures have become a great concern, and have forced nutritionists to create dietary protein that meets the AA balance required by animals at metabolic level more closely. Since the start of synthetic AA production, breakthroughs have been made with supplementation of first limiting AAs to diets.

Today, most indispensable AAs can be obtained in the crystalline form. Addition of these AAs firstly allows for reduced dietary crude protein (CP) inclusion rates and secondly supplies the animal with an AA balance that is closer to that required by the animal. This approach has been reported to improve efficiency of nitrogen utilisation and consequently to reduce nitrogen excretion (Dourmad *et al.,* 1999). Decreasing the CP content of the feed not only results in a reduction in nitrogen pollution to the environment, but also in the production of a more economical feed. Additionally, the severity and incidence of digestive upsets are reduced (Ball & Aherne, 1987). Lysine, methionine, threonine and tryptophan are generally considered the first four limiting AAs in maize-soybean based diets for pigs. Experiments conducted by Figueroa *et al.* (2003) showed that valine is the next limiting AA. Results of Mavromichalis *et al.* (1998) indicated that after lysine, valine is a second co-limiting AA with methionine, threonine and tryptophan in a low protein maize-soybean meal-whey-based diet (13.5% CP) for weaned piglets (10kg). A consensus is that valine is the fifth limiting AA in maize-soybean based diets and that after supplementation of lysine, methionine, threonine and tryptophan to the diet, a marginal deficiency in valine will limit the performance of pigs.

In the past, valine supplementation was economically unfeasible, but has become competitive in weaner diets due to advances in fermentation technology (Gaines *et al.,* 2011). There have been a few attempts to determine the optimal level of valine in the diet of growing pigs. Chung & Baker (1992) estimated an optimal valine to lysine ratio of 0.68.

Recent estimates of standardised ileal digestible (SID) valine to lysine ratios reported for pigs of body weight 12-25 kg, 8-25 kg, 13-32 kg and 6.8-11.36 kg are 0.68-0.80, 0.65-0.67, 0.65 and 0.647, respectively (Barea *et al.*, 2009; Wiltafsky, 2009; Nemecheck *et al*., 2010; Gaines *et al*., 2011).

In all the above experiments, relating to the response of weaned piglets to valine, the traditional method of measuring responses was used, namely, the graded supplementation technique. This method involves the use of a basal diet severely deficient in the AA under test, which is then supplemented with the test AA in increasing doses to obtain a range of levels of test AA. A dose-response curve is produced to establish at which inclusion rate growth rate is optimal. However, this technique poses a few difficulties. The major criticism of this technique is concerned with the change in AA balance created when the test AA is successively increased in the supplementation series (Gous & Morris, 1985). A summit dilution technique was proposed by Fisher & Morris (1970) to overcome these complications. This method consists of diluting a high protein diet (summit mix) with a protein-free and iso-energetic diet (dilution mix) in different proportions to obtain a series of diets. This method makes the AA under test first-limiting, ensures identical AA balances throughout the series and generates a method of measuring the response to one essential amino acid (EAA). Thus, there are no confounding effects between the AA balance and the level of the first-limiting AA, and the AA under test remains first-limiting throughout the series. The summit dilution technique may therefore give more accurate results than the graded supplementation technique when determining AA requirements.

Valine, leucine and isoleucine share common structural characteristics and are classified as branched chain amino acids (BCAAs). BCAAs share common systems for transport through cell membranes and use the same enzymes for degradation. A high leucine intake stimulates the activity of branched-chain α-keto acid (BCKA) dehydrogenase which is the enzyme involved in the breakdown of all three BCAAs (Harris *et al*., 2001; Baker, 2005). The resultant increased oxidation of valine and isoleucine causes a deficiency in these AAs and limits pig performance (Smith & Austic, 1978). High plasma leucine levels indicate to the animal that protein intake was sufficient and that feed intake can stop (Wiltafsky, 2009). Food intake regulation is the homeostatic response to regulate the AA concentration in plasma and tissues by reducing the influx of AAs (Harper, 1974). Some feedstuffs used for pig diets, for example

dried distiller's grains and solubles (DDGS), maize and maize gluten contain high leucine contents relative to other AAs and may cause an oversupply of this AA in the diet. This could potentially limit growth and production due to the detrimental effect on feed intake.

This trial is distinctive in that valine requirements are determined using the dilution technique as opposed to the graded supplementation method. Additionally, most trials have focused on effects on growth rates whereas this study aims at measuring effects on carcass characteristics such as protein and lipid growth. Various studies confirm interactions between dietary BCAAs, especially when leucine is fed in excess (D'Mello & Lewis, 1970; Edmonds & Baker, 1987; Gatnau *et al*., 1995). Edmonds & Baker (1987) and Gatnau *et al.* (1995) stated that diets containing 6% leucine (about 2.5 times the amount required by piglets) decreased pig performance. D'Mello & Lewis (1970) and Henry *et al.* (1976) showed that a valine-limiting diet together with an excess leucine concentration in the feed is more detrimental to pig performance than a diet limiting in isoleucine. However, Langer & Fuller (2000) found that pigs fed diets limiting in isoleucine were more affected than valine-limiting diets when leucine was given in excess of requirement. Therefore, more studies need to confirm the concentration of leucine where pig performance is being negatively affected and also if a valine-limiting diet containing excess leucine will cause a depression in pig performance. When a depression in performance occurs, it should be evaluated if valine supplementation counteracts such depression.

The aim of this trial was to determine the response in feed intake, growth and carcass composition of weaned piglets to changes in dietary valine and leucine content. It is hypothesised that high levels of dietary leucine will have a negative effect on feed intake, growth and carcass composition, but that this can be counteracted by increasing the valine content of the feed.

CHAPTER 2

LITERATURE REVIEW

2.1 THE REQUIREMENT FOR AMINO ACIDS

2.1.1 Protein and amino acids

The protein level in feed, generally known as crude protein (CP), is measured as the nitrogen content of mixed feedstuffs x 6.25, which is derived from the assumption that, on average, 100g of protein contains 16g nitrogen. Protein is comprised of a collection of peptides which are composed of amino acids (AAs) (NRC, 1998). Proteins are digested enzymatically in the stomach and small intestine to peptides and AAs which are absorbed by intestinal cells. Therefore, an animal does not have a specific requirement for CP, but rather a specific need for individual AAs. Pigs require AAs from food for anabolism of muscle tissue and to replace proteins lost by turnover of protein tissue (maintenance) which are voided in cells, AAs and unabsorbed enzyme secretions from the intestine (also known as endogenous or metabolic faecal losses). Pigs also require AAs to construct a range of non-protein compounds such as hormones, neurotransmitters, immunoglobulins and replacement of proteins lost as hair and skin or from the gastrointestinal tract (Whittemore & Kyriazakis, 2006).

2.1.2 Essential and nonessential amino acids

There are currently more than 20 known AAs needed as building blocks for proteins. Some AAs can be synthesised from carbon skeletons such as glucose and other AAs present in excess of requirement. Such AAs are called nonessential amino acids (NEAAs) or dispensable AAs. AAs that are not synthesised by the body or are synthesised at a rate that will not satisfy cellular demand for growth or reproduction are defined as essential amino acids (EAAs) or indispensable AAs. Therefore, pig nutrition is focused on supplying EAA in amounts directly proportional to the amounts required by pigs. Some AAs cannot be classified as either EAA or NEAA, and are called conditional EAAs. These are arginine, cysteine, phenylalanine and glutamine. Arginine is synthesised in the body at a sufficient rate in post pubertal and pregnant pigs, but is not synthesised at a sufficient rate in growing and lactating pigs (NRC, 1998). Cysteine can be synthesised from methionine and is thus a NEAA. Although methionine

cannot be synthesised from cysteine, cysteine and its oxidation product, cystine, can satisfy approximately 50 percent of total sulphur AA requirement and can reduce the requirement for methionine (Chung & Baker, 1992). Phenylalanine can be converted to tyrosine, but not the other way round. Tyrosine can meet at least 50 % of the requirement for both phenylalanine and tyrosine, but cannot serve as the only source. Glutamine is a conditional EAA because it prevents intestinal atrophy in some circumstances (NRC, 1998). Pigs can use dietary AAs as an energy source after deamination of the AAs. The EAAs and NEAAs known to be required for growth in pigs are shown in Table 2.1.

Table 2.1 Essential and nonessential amino acids required in pigs (Campbell & Farrel, 2006)

Essential amino acids	Nonessential amino acids
Lysine	Alanine
Methionine	Asparagine
Tryptophan	Aspartic acid
Threonine	Cysteine
Valine	Glutamic acid
Isoleucine	Glycine
Leucine	Proline
Histidine	Citrulline
Phenylalanine	Serine
Arginine	Tyrosine

2.1.3 Amino acids in pig diets

Cereal grains such as maize, wheat, barley or sorghum are primary ingredients of most pig diets and normally provide 30-60% of total AA requirements. However, other protein sources such as soybean meal have to be included in the diet to ensure adequate intakes of EAAs. The level of protein sources to be included to ensure adequate EAA intake will depend on the quality of feed proteins. A good quality protein has an AA composition relatively similar to the requirement of the pig. Feed ingredients can complement each other where the overall AA composition of the mixture closely resembles the pig's requirement (NRC, 1998). To incorporate the feed AAs into body proteins, feed protein must first be broken down to AAs, the AAs must be absorbed and body proteins be synthesised. Excess AAs must be deaminated

and the nitrogen excreted as urea. All of these processes require relatively high levels of energy (Whittemore & Kyriazakis, 2006).

2.1.4 The ideal protein concept

Dietary protein quality is determined by AA profile, availability and digestibility. Protein quality can be considered as the extent to which the composition of the absorbed AA mixture corresponds with the balance required by the animal (Wang & Fuller, 1989). The more closely a balance of absorbed AA conforms to the requirements of an animal, the higher is its biological value. An ideal protein can be described as one that has a perfect AA balance required for a specific need or function within the animal. An ideal protein supplies the optimum balance of EAAs together with adequate nitrogen for the synthesis of NEAAs. If pig feed contains a protein composed of the precise amount of each EAA needed by the pig, it theoretically is an ideal protein and each EAA is equally limiting. Lysine is one of the 10 indispensable AAs and is mainly used for protein synthesis. Lysine is usually the first limiting AA, in other words the most deficient relative to the requirement of pigs and is most often used as a reference AA. Generally, AA requirements are expressed as a percentage relative to lysine. This simplifies diet formulation as well as evaluation of AA patterns of feedstuffs (Wiltafsky, 2009). Today, feeds are often formulated to supply an excess of protein as a safety margin to ensure maximum pig growth performance. Oversupplementation of pig diets makes the ration more expensive and increases the amounts of nutrients excreted into the environment. Nitrogen, copper, zinc, phosphorus, potassium and sodium are the minerals that cause the greatest concern (NRC, 1998). Due to the increasing availability of crystalline AAs, it is possible to decrease the CP level in pig diets when accompanied by correct AA supplementation (Kerr *et al.*, 1995; Figueroa *et al.*, 2002; Lordelo *et al.*, 2008). Kerr *et al.* (1995) found that a reduction of dietary CP in growing pigs by four percent units severely reduced pig performance and carcass leanness without AA supplementation. However, when the diets were supplemented with AAs, weight gain was similar between pigs fed reduced CP, AA supplemented diets and those fed the recommended dietary CP levels. Figueroa *et al.* (2002) showed that the protein content of maize-soybean based diets could be reduced from 16 % to 12% for pigs weighing 20-50 kg without a drop in growth performance if crystalline lysine, tryptophan, threonine and methionine were supplemented to the diet. Feeding a diet containing an AA balance as close as possible to the requirement of pigs reduces nitrogen excretion. Kerr & Easter (1995) found that every one percentage unit reduction in CP

combined with the proper AA supplementation, resulted in an approximate 8% drop in total nitrogen loss (faecal plus urinary). The protein component of a ration is normally the most expensive component and if this part of the ration can be lowered, a more economical feed can be produced.

2.1.5 Amino acid imbalance

Valine, leucine and isoleucine form part of three BCAAs as described in more detail in the next section. Addition of crystalline valine to pig diets now raises the issue of an imbalance with leucine because it is well known that there exists an antagonistic relationship between the BCAAs in monogastric animals (D'Mello & Lewis, 1970; Burnham *et al.*, 1992; Gatnau *et al.*, 1995; Baker, 2005).

Dried distiller's grains with solubles (DDGS) is a byproduct from the fuel ethanol and beverage industries. It consists mainly of components such as protein, fat and fibre which are concentrated approximately three times after the grain starch has been removed and fermented to alcohol. Thus, diets containing high levels of DDGS will contain higher CP concentrations than maize-soybean based diets (Williams *et al*., 2010). DDGS has been successfully included in pig diets and is a good source of energy, minerals, protein and vitamins. Whitney & Shurson (2004) have successfully included DDGS at a 25% level in diets of weaned piglets without any negative effect on growth performance, but found that piglets weighing less than 7 kg should not receive high levels of DDGS as it depresses feed intake and growth. Williams *et al.* (2010) found that an inclusion of 30% DDGS in diets of weaned piglets had no significant effect on average daily gain (ADG) or average daily feed intake (ADFI), but affected feed:gain (F/G) negatively. Any inclusion of more than 30% DDGS has been reported to reduce growth performance (Williams *et al*., 2010). Due to these high inclusion rates of DDGS that contain relatively high concentrations of leucine, there is a high risk of feeding excess leucine to pigs, in relation to relative low valine levels. This raises the issue of an imbalance between valine and leucine. Table 2.2 shows the AA concentrations of DDGS.

Table 2.2 Essential amino acid composition (%) of dried distillers grains with solubles

Amino Acid	Stein (2007)[a]	Cromwell *et al.* (1993)[b]	Shurson *et al.* (2004)	Batal & Dale (2006)[c]	AminoDat 3.0[d]	Avg.	% of required[e]
Lysine	0.78	0.70	0.85	0.71	0.74	0.756	108
Methionine	0.55	0.51	0.55	0.54	0.51	0.532	280
Threonine	1.06	1.03	1.13	0.96	0.99	1.034	246
Tryptophan	0.21	0.19	0.25	1.09	0.21	0.390	300
Valine	1.35	1.35	1.50	1.33	1.27	1.360	283
Histidine	0.72	0.72	0.76	0.69	0.69	0.716	325
Isoleucine	1.01	1.00	1.12	0.97	0.97	1.014	267
Phenylalanine	1.34	1.45	1.47	1.31	1.29	1.372	327
Leucine	3.17	3.33	3.55	3.05	3.05	3.230	455
Arginine	1.16	1.06	1.20	1.09	1.15	1.132	333

[a] Concentration of amino acids in 36 samples of DDGS.
[b] Concentration of amino acids in 9 samples of DDGS.
[c] Concentration of amino acids in 8 samples of DDGS.
[d] AminoDat is a registered Trademark of the Degussa AG. Values represent mean AA % of dried distiller's grains with solubles.
[e] Average percentage of amino acid composition of protein in DDGS relative to that required for purposes of satisfying the daily rate of protein retention in weaned piglets (ARC 1981; NRC 1998).

Maize is also known to contain a slight excess of leucine as illustrated in Table 2.3.

Table 2.3 Essential amino acid content (%) of maize

Amino Acid	Belyea *et al.* (2004)	AminoDat 3.0[a]	Avg.	% of required[b]
Lysine	0.24	0.25	0.245	35
Arginine	0.54	0.39	0.465	137
Threonine	0.39	0.30	0.345	82
Valine	0.51	0.38	0.445	93
Isoleucine	0.39	0.28	0.335	88
Histidine	0.25	0.24	0.245	111
Methionine	0.21	0.17	0.190	100
Phenylalanine	0.49	0.40	0.445	106
Tyrosine	0.43	0.24	0.335	-
Tryptophan	0.09	0.06	0.075	58
Leucine	1.12	1.00	1.060	149

[a] AminoDat is a registered Trademark of the Degussa AG. Values represent mean AA % in maize.
[b] Average percentage of amino acid composition of protein in maize relative to that required for purposes of satisfying the daily rate of protein retention in weaned piglets (ARC 1981; NRC 1998).

Maize gluten meal (MGM) is often fed to pigs as a source of protein, but one of the major shortcomings of feeding MGM is that it has a severely imbalanced AA profile which is shown in Table 2.4. It is evident from Table 2.4 that leucine content of maize gluten is relatively excessive.

Table 2.4 Essential amino acid composition values (%) of maize gluten meal

Amino Acid	(Peter *et al.*, 2000)[a]	AminoDat 3.0[b]	Avg.	% of required[c]
Lysine	0.98	0.98	0.98	140
Arginine	2.03	1.89	1.96	576
Threonine	2.00	2.01	2.01	477
Valine	2.68	2.72	2.70	563
Isoleucine	2.58	2.38	2.48	653
Histidine	1.19	1.26	1.23	557
Methionine	1.46	1.46	1.46	768
Phenylalanine	4.31	3.74	4.03	958
Tyrosine	2.80	3.39	3.10	1190
Leucine	10.87	9.75	10.31	1452

[a] Average of duplicate determinations. Values are expressed as a percentage of as-fed maize gluten meal (90.9% Dry Matter).
[b] AminoDat is a registered Trademark of the Degussa AG. Values represent mean AA % in maize gluten.
[c] Average percentage of amino acid composition of protein in maize gluten relative to that required for purposes of satisfying the daily rate of protein retention in weaned piglets (ARC 1981; NRC 1998).

2.1.6 Valine in pig nutrition

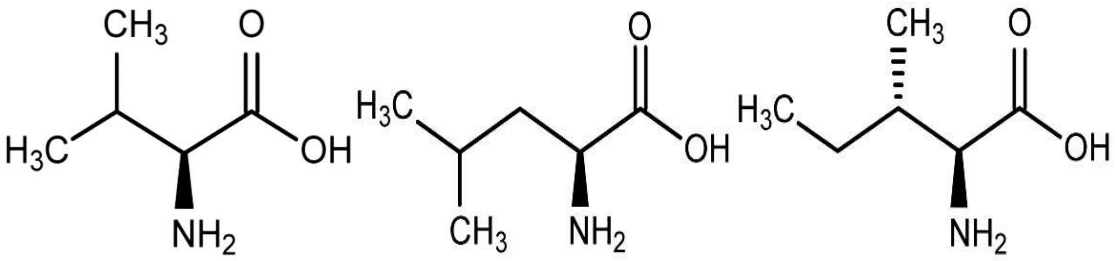
Figueroa *et al.* (2002) showed that the protein content of a diet could be lowered from 16 % to 12% with adequate supplementation of the first four limiting crystalline AAs (lysine, methionine, threonine and tryptophan), but when the protein content was lowered to 11% there was a significant drop in growth and feed efficiency because other EAAs became limiting. Their research indicated that histidine, isoleucine and valine were most likely to be the next limiting. In a subsequent experiment, Figueroa *et al.* (2003) found that valine was fifth limiting and that either isoleucine or histidine was sixth limiting. Russel *et al.* (1987) concluded from the results of their experiments that valine is the only limiting AA when a maize-soybean based diet not limiting in sulphur AAs and supplemented with lysine, tryptophan and threonine is fed to growing pigs. These results strongly suggest that valine is fifth limiting in a diet adequately fortified with the other four limiting AAs in crystalline form.

Reductions in CP content not only reduce nitrogen output and ground water pollution with nitrogen, but also reduce the severity and incidence of digestive upsets (Ball & Aherne, 1987). Since the recent start of production of synthetic valine, there has been considerable interest in measuring the response to increasing valine intake to determine optimal levels in feed.

2.2 BRANCHED CHAIN AMINO ACIDS (BCAAs) AND THEIR FUNCTIONS

2.2.1 Description of BCAAs

Valine, leucine and isoleucine are classified as BCAAs based on similar structural qualities (Fig. 2.1). Lysine, methionine, threonine and tryptophan are first limiting in most commercial diets and the BCAAs are classified as secondary limiting, but this is only because of the types of ingredients commonly used in pig feeds. The order of limitation is dependent on the requirement of the animal for each AA the concentration of these in the feed ingredients used.



L-Valine L-Leucine L-Isoleucine

Figure 2.1 Chemical structures of the branched chain amino acids

2.2.2 Functions of BCAAs

The BCAAs are important precursors for AA and protein synthesis. Animals cannot synthesise valine, leucine and isoleucine. Together they make up approximately 35% of the EAAs in muscle proteins and approximately 40% of the AAs required by mammals (Harper *et al.*, 1984). The BCAAs are important energy substrates in the brain. Even when other energy substrates like glucose are abundant, the BCAAs are rapidly catabolised to branched chain keto acids which are released into the extracellular fluid where the neurons take them up and

metabolise them (Platell *et al.*, 2000). They also serve as regulators of protein degradation and biosynthesis and secretion of insulin of which leucine is most efficient at regulating these processes. High dietary leucine levels can influence insulin release (Harper *et al.*, 1984). In skeletal muscle, leucine has the unique role of up-regulating several steps in mRNA translation which results in a rapid increase in protein synthesis (Kimball & Jefferson, 2006). Endogenous BCAAs serve a major role as a nitrogen source for the *de novo* synthesis of alanine. Following an overnight fast, 20% of the nitrogen required for alanine synthesis is derived from leucine and valine (Platell *et al*., 2000). Abumrad *et al*. (1982) examined the effect of leucine infusion on AA and carbohydrate flux across the human forearm. They found that infusion of leucine decreased circulating levels of the other BCAAs, valine and isoleucine, and also methionine and tyrosine. They also found that leucine directly caused a decrease in peripheral glucose utilisation and, together with an indirect influence through increasing insulin levels, suppressed liver glycogenesis and hence caused a drop in blood glucose and elevated blood lactate levels. During starvation, the muscles are in a catabolic state, which causes the release of endogenous BCAAs, which are the primary precursors for the *de novo* synthesis of glutamine (Darmaun & Dechelotte, 1991). The BCAAs are substrates for the synthesis of glutamine, which is unstable in solutions and therefore often deficient when conventional diets are fed. Glutamine serves an important role during cell replication, especially in the immune system and in the gut. When diets are deficient in glutamine, gut atrophy and immune diseases often prevail (Platell *et al.*, 2000). Parenteral infusion of BCAAs in protein-undernourished rats caused an increase in plasma glutamine concentration (McCauley *et al.*, 1991). Ferrando *et al.* (1995) studied the effects of BCAA ingestion in normal men and found that BCAA ingestion suppresses whole-body protein degradation in tissues excluding skeletal muscle. During prolonged and sustained heavy exercise, BCAA uptake and oxidation by the working muscle is increased because BCAAs serve as an energy substrate and after an increased supply of BCAAs during exercise, muscle glycogen degradation is lowered, creating a sparing effect on muscle glycogen (Ferrando *et al*., 1995; Blomstrand *et al.*, 1996).

2.2.3 Types of adverse effects

It is essential to distinguish between the terms toxicity, antagonism, imbalance and deficiency when considering the negative effects resulting from feeding diets containing disproportionate amounts of AAs. Harper (1974) described these terms as following: A deficiency occurs when

there is an inadequate amount of AA taken in. An imbalance is dissimilar from toxicity in that toxicity relates to a condition in which a negative effect is caused by an excess of AAs other than the first limiting AA. If the AAs are imbalanced, the total amount of AAs in the diet may be greater than the amounts causing toxicity, but no single AA is included in the diet in an amount that would, on its own, be considered toxic. Edmonds & Baker (1987) report that a large surplus of arginine does not antagonise lysine, but rather causes an AA imbalance. Antagonism occurs between AAs that are structurally related such as the BCAAs which will be described more in the next section.

2.2.4 Effects of excess BCAAs (Imbalance)

Burnham *et al*. (1992) studied the effects of excess leucine and valine on the response to isoleucine. They carried out three experiments to determine the response of broiler chickens to dietary isoleucine and to quantify the antagonistic effects of excess leucine and valine on this response. The most important conclusions they made from these experiments are that valine did not interact with isoleucine over the range of isoleucine concentrations; that an increase in the leucine to isoleucine ratio resulted in poorer growth, but only at the lowest concentrations of isoleucine; and that this poorer growth was caused by an imbalanced diet, which resulted in a depressed feed intake. Harper *et al.* (1970) recognised antagonisms between BCAAs and identified the main effect of an imbalanced feed AA balance to be on FI.

When the isoleucine requirements of the broiler are met by the isoleucine content of the feed, relatively large surpluses of leucine and valine can be tolerated by the animal and will not depress growth. Baker (2005) fed pigs a diet containing about six times the required level of leucine and found that this level of surplus leucine caused pigs to deposit more fat in muscle compared to the control level. D'Mello & Lewis (1970) carried out experiments on chicks to study the interrelationships between leucine, isoleucine and valine. They found that the performance of animals is depressed when leucine is added in excess in the diet, but the effect is prevented if the diet is appropriately supplemented with isoleucine and valine. In another experiment, D'Mello & Lewis (1970) showed that supplementing the diet with valine lowers the leucine levels in plasma following the addition of excess leucine. The level of valine required to alleviate the negative effects caused by excess leucine levels in the diet depends on the degree of surplus leucine in the diet. Isoleucine supplementation only partially removed the adverse effects. Leucine is able to exert an influence on the fate of both leucine and

isoleucine at the same time, which is the reason for the incomplete reversal by isoleucine of the negative effects caused by an excess leucine in the diet. This clarifies why valine is more sensitive to the presence of excess leucine in the diet than isoleucine and that there is a clear leucine-valine interaction.

The leucine-valine interaction has unique properties in relation to the interrelationship between leucine and isoleucine and is more capable to disturb the equilibrium of the BCAAs. Interrelationships similar to those above have been observed with rat experiments (Harper *et al*., 1954). Experiments, carried out by Edmonds & Baker (1987) to study the effects of excess methionine, tryptophan, threonine or leucine, showed that the performance of young pigs was decreased when leucine was included in the diet at 6%, which is about 2.5 times the amount required by the pig, but probably is not harmful. They also found that surplus leucine decreased plasma isoleucine and valine levels, an indication of antagonism. They also stated that levels of valine and isoleucine in maize-soybean meal pig diets are above the requirement to prevent antagonism of BCAAs. Gatnau *et al*. (1995) did experiments to study effects of excess dietary leucine on growth and immune responses in weaned piglets. They also observed growth depressions in pigs fed the basal diet supplemented with 6% leucine and results of plasma free AAs proved that excessive leucine intakes from the diet increased plasma leucine and decreased plasma isoleucine and valine. Their results once again verify the antagonistic relationship between the BCAAs. They implied that typical leucine concentrations in pig weaner diets based on maize and soybean are unlikely to cause harmful effects, but leucine concentrations double the amounts found in practical diets are likely to cause reduced growth performance and immune function.

Peganova & Eder (2003) studied the interactions of BCAAs to see how these affect the performance of laying hens. At low dietary valine and leucine concentrations, an increase in the isoleucine concentration led to a significant reduction in performance parameters. At high dietary valine + leucine concentrations, an increase in the isoleucine concentration had less effect on performance parameters. Thus, the effect of isoleucine concentration in the diet on levels of plasma isoleucine levels is dependent on the dietary level of valine and leucine. The dietary isoleucine concentration has no effect on the concentrations of leucine and valine in plasma. Increasing the dietary valine and leucine concentrations leads to a significant increase in concentrations of those AAs. The experiment validates the existence of antagonism between the three BCAAs. Henry *et al*. (1976) also studied the leucine-isoleucine interrelationship.

Increasing either of the two AAs in the diet resulted in an increase in both of the AAs in blood. However, when leucine in the diet was increased, valine and isoleucine concentrations in the blood decreased, resulting in an overall decrease of the total free AAs in blood. In their study, no leucine-isoleucine antagonism, as reflected by a deleterious effect on growth and feed intake, was found after almost doubling the leucine–isoleucine ratio. They concluded that under usual feeding conditions where diets are based on maize, there is no justification for correcting the isoleucine allowance for leucine level. In contrast, Langer & Fuller (2000) did an experiment on pigs and found that diets limiting in isoleucine were more affected by an excess of leucine than valine-limiting diets, indicating that leucine affected the utilisation of isoleucine more than that of valine. However, most experiments reveal that excess leucine has a more detrimental effect on growth performance when the diet is valine limiting rather than isoleucine-limiting.

Following from the literature described above, it can be concluded that an excessive level of leucine in the feed causes an antagonistic response against valine and isoleucine.

2.2.5 Physiological and metabolic responses to excess BCAAs

Animals fed a low protein diet have reduced activities of enzymes responsible for AA catabolism, thereby limiting their capacity to remove excess ingested AAs. The surplus AAs cannot be used to synthesise tissue protein. Under these conditions, AA degrading enzymes do not undergo adaptation as they would do in animals fed a high protein diet. If the ingestion of such diets continues to exceed the capacity of AA degradation, AAs will continue to accumulate in body fluids whereafter toxic levels may be reached unless some regulatory mechanism comes into play. Food intake regulation is the homeostatic response to regulate the AA concentration in plasma and tissues by reducing the influx of AAs (Harper, 1974). The three BCAAs are structurally similar, share common systems for transport through cell membranes and use the same enzymes for degradation. A major factor causing antagonistic effects is the competition between the three AAs for transfer into the brain across the blood-brain barrier. An excessive intake of isoleucine may result in an increase in isoleucine concentrations in the blood, which may lead to depletion of other structurally similar AAs in the brain, causing secondary anorexia. A decrease in feed intake then leads to a depressed performance (Peganova & Eder, 2003).

At high dietary valine and leucine concentrations, surplus isoleucine supplied through the diet did not cause a significant increase in blood plasma isoleucine level. Peganova & Eder (2003) suggested that this is due to increased oxidation of leucine, isoleucine and valine as a result of the increased leucine intake. A high leucine intake stimulates the activity of branched-chain α-keto acid (BCKA) dehydrogenase which is the enzyme involved in the breakdown of the BCAAs (Harris *et al*., 2001; Baker, 2005). Increased oxidation of valine and isoleucine causes deficiency in these AAs, which results in a performance decreasing effect (Smith & Austic, 1978). Surplus dietary isoleucine does not stimulate BCKA dehydrogenase activity because at normal or slightly higher intakes of valine and leucine, the concentrations of these AAs in plasma are independent of the isoleucine concentration in the diet (Peganova & Eder, 2003). Also in rats, a high isoleucine intake does not enhance valine and leucine oxidation (Block *et al*., 1985). Oestemer & Hanson (1973) concluded that although leucine inhibits the absorption of valine and isoleucine in the upper portion of the intestine, the ratio of leucine to the other two AAs will change as ingesta moves to the lower regions of the small intestine. Thus it is likely that competition between BCAA at absorption sites does not affect absolute amounts of isoleucine and valine absorbed. Leucine appears to function as a nutrient signal that tells the organism about the protein intake. Thus, high plasma leucine levels indicate the organism that protein intake was sufficient and that intake of feed can be stopped (Wiltafsky, 2009).

2.2.6 Metabolism of BCAAs and branched-chain α-ketoacid (BCKA)

Harper *et al.* (1984) described the metabolism of BCAAs and BCKA as follows. Because the BCAAs have similar structures, they have few features in common and therefore also follow similar catabolic pathways. Each BCAA undergoes transamination to produce the corresponding BCKA. BCAA aminotransferase catalyses the transamination reaction and recognises all three BCAAs as substrates. Each BCKA is converted to an acyl-CoA derivative and CO_2 through an oxidative decarboxylation reaction catalysed by BCKA dehydrogenase. The pathways that follow are similar to those for fatty acid oxidation, which yield end products that can enter the Krebs cycle. The enzymes involved in BCAA catabolism are unique because they are distributed throughout the body and not restricted to the liver. Valine catabolism yields succinyl-CoA and is therefore glucogenic. Leucine degradation yields acetoacetate and acetyl-CoA and is therefore ketogenic. Isoleucine catabolism yields propionyl-CoA and acetyl-CoA and is therefore both ketogenic and glucogenic.

2.3 MEASURING THE RESPONSE TO VALINE INTAKE USING THE SUMMIT DILUTION TECHNIQUE

The graded supplementation method is most commonly used to measure responses to AAs. This technique involves feeding a basal diet, which is severely limiting in the AA under test, alone and with increasing amounts of the test AA in synthetic form. Various methods are then used to determine the requirement of the AA from the derived response curve (Gous & Morris, 1985). However, this technique poses a few difficulties. Firstly, by continuously adding the limiting AA to the basal diet deficient in that AA, the AA balance is not the same in each diet which may affect the results. Secondly, it is difficult to put together a basal diet that is adequately deficient in the test AA but sufficient in all others which makes it possible to examine only a narrow range of input levels. Thirdly, at high supplementation levels the AA being tested may no longer be first-limiting and there might be an additional response if new first-limiting AAs were supplemented to the diet (Fisher & Morris, 1970; Gous & Morris, 1985). Fisher & Morris (1970) suggested the use of a summit dilution technique which is a formulation technique that can overcome these disadvantages to a large extent. They developed this technique originally to measure the response of laying hens to increasing intakes of methionine. This method involves the dilution of a summit mix or a high protein diet with a protein-free and isoenergetic diet in different proportions to obtain a series of diets. The summit mix must contain all AAs in excessive amounts (for example not less than 1.80 of the assumed requirement), except the AA under experiment needs to be included at a lower concentration (for example 1.40 of assumed requirement) (Gous & Morris, 1985). This method causes the AA under test to be first-limiting and generates a method of measuring the response to one EAA (Fisher & Morris, 1970). Morris *et al.* (1999) carried out experiments that yielded results showing that growth is a function of the AA intake under test and that protein intake seems to be irrelevant up to a point where a concentration of 300 g CP/ kg is reached which justified the use of the dilution method. Although evidence proved that protein level could have an effect on the estimated AA response curve (but not in every situation), results of the dilution method will provide estimates of response that are closely related to estimates required in feed formulation. Fisher & Morris (1970) showed that the response to the test AA obtained is virtually independent of the level of protein and is not influenced by sequential dilution of the summit feed. Williams *et al.* (2010) studied the effects of feeding excessive levels of protein to weaned piglets. Their treatments consisted of three levels of CP: 22.5 %, 25 % and 27.5 %. Increasing the % CP of the diet from 22.5 to 27.5 % had no effect

on average daily gain (ADG), average daily feed intake (ADFI) or feed to gain ratio (F/G). The aim of sequentially diluting a high protein diet relatively deficient in the AA under test with a protein-free, isoenergetic diet is to provide or maintain a constant AA pattern throughout all treatments because the concentration and ratio of EAAs and NEAAs may directly influence the ability of animals to efficiently use AAs (Gous, 2010). Due to the need of a more practical number of treatments, the dilution method will more likely yield realistic estimates of the response to the AA under test than the graded supplementation technique (Morris *et al*., 1999).

2.4 REPORTED VALINE REQUIREMENTS IN GROWING PIGS

Philosophically, the so-called AA requirement trials reported in the literature are actually response trials which are used to determine the 'requirement' of the animal for that amino acid. Because the shape of the response is curvilinear there can be no such concept as a 'requirement' because the response is measured over a period of time which introduces some curvature to the shape of the response, making it necessary to define what is meant by the requirement. There are many such definitions (Morris, 1999), the most rewarding of which is when the marginal cost of the AA and the marginal revenue generated by the sale of the product are used to determine the level of AA in the feed that would generate the highest profit for the enterprise. One such method is commonly known as the Reading Model (Fisher *et al*., 1973). However, other methods based on statistical techniques, are often used making it difficult to compare 'requirements' that have been published in the literature.

Almost every trial conducted to determine the optimal valine requirements for growing pigs used the graded supplementation technique and valine requirements are expressed as % valine in the diet, valine to lysine ratio, apparent ileal digestible (AID) valine, standardised ileal digestible (SID) valine/ MJ of ME, g AID valine/ MJ of ME, g of valine/ day and % SID valine. Reported valine requirements in growing pigs are shown in Table 2.5.

Table 2.5 Reported valine requirements in growing pigs

Reference	Body weight range (kg)	Valine requirement
Mavromichalis *et al.* (2001)	5 to 10	0.60 of SID[#] Val/MJ of ME
Mavromichalis *et al.* (2001)	10 to 20	0.53g of SID Val/MJ of ME
Theil *et al.* (2004)	8 to 20	0.59g of AID[+] Val/MJ of ME
Lewis & Nishimura (1995)	67 to 80	11.4g of SID Val/day
Gaines *et al.* (2011)	13 to 32	SID Val:Lys of 65%
Barea *et al.* (2009)	12 to 25	SID Val:Lys of 68 to 80%
Chung & Baker (1992)	Not specified	SID Val:Lys of 68%
Wiltafsky (2009)	8 to 25	SID Val:Lys of 65 to 67%
Nemecheck *et al.* (2010)	6.8 to 11.36	SID Val:Lys of 64.7%

[#]SID: Standardised ileal digestible valine
[+]AID: Apparent ileal digestible valine

Figure 2.2 shows the relationship between standardised ileal digestible (SID) valine % of feed and average daily gain for piglets of different weight ranges from different authors.

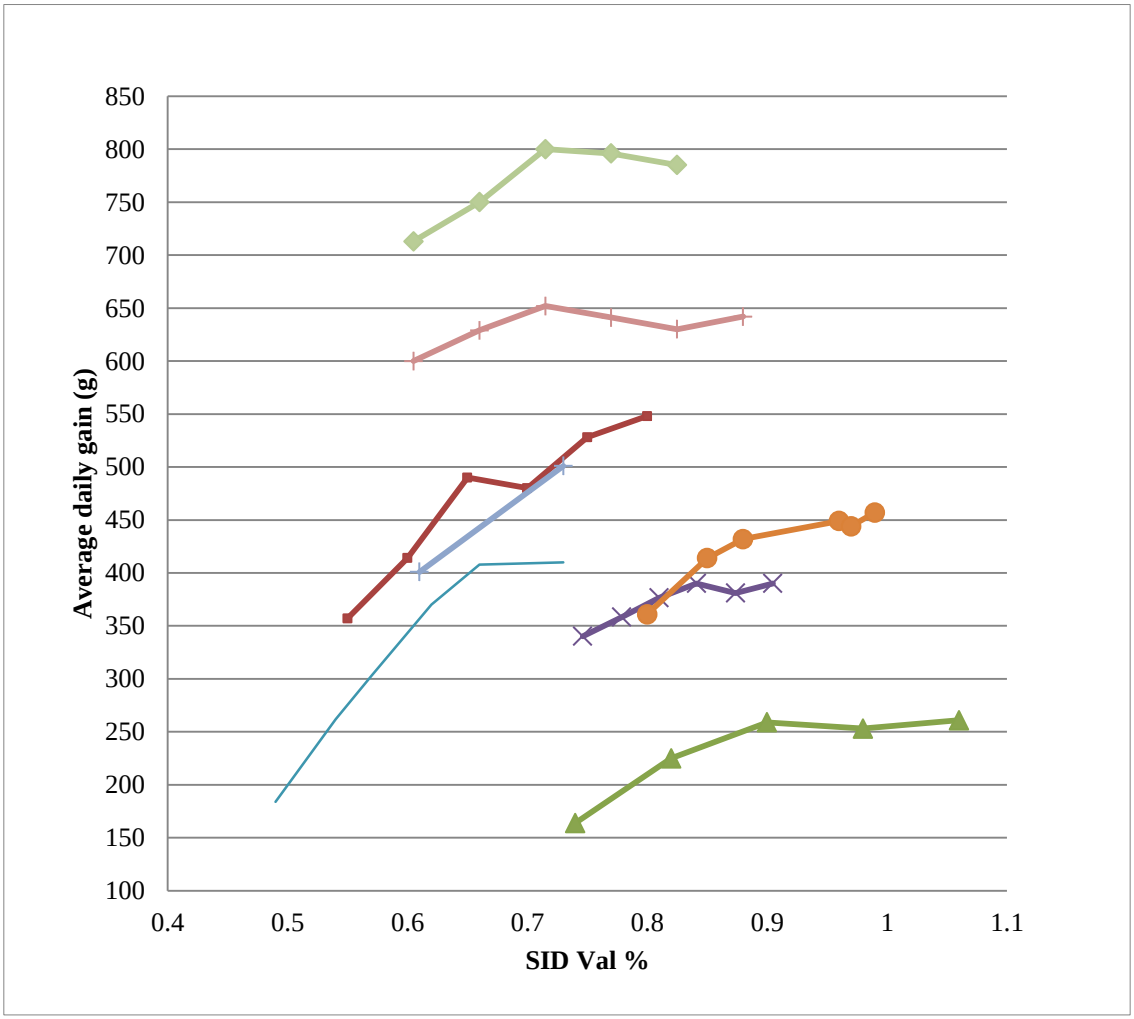


Figure 2.2: Reported relationships between standardised ileal digestible (SID) valine % of feed and average daily gain (ADG) for (a) Mavromichalis *et al*. (2001) piglets 5-10 kg BW (▲), (b) Wiltafsky (2009) piglets 8-25 kg BW (–), (c) Mavromichalis *et al*. (2001) piglets 10-20 kg BW (■), (d) Nemecheck *et al*. (2010) piglets 6.8-11.36 kg BW (×), (e) Theil *et al*. (2004) piglets 8-20 kg BW (●), (f) Barea *et al*. (2009) piglets 12-25 kg BW (+), (g) Gaines *et al*. (2011) piglets 13-27 kg BW (+), (h) Gaines *et al*. (2011) piglets 21-32 kg BW (♦).

Figure 2.3 shows the relationship between standardised ileal digestible (SID) valine % of feed and feed intake for piglets of different weight ranges from different authors.

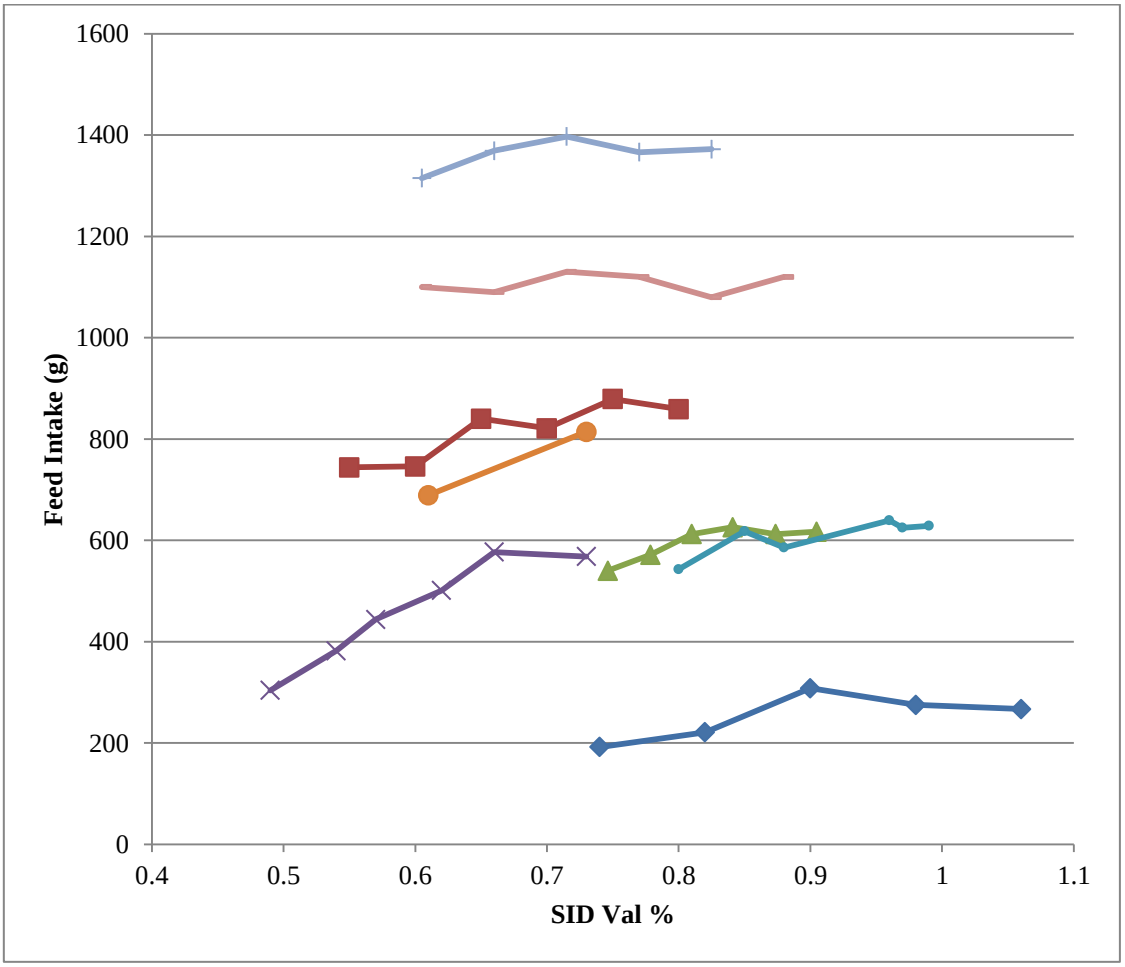


Figure 2.3: Reported relationships between standardised ileal digestible (SID) valine % of feed and feed intake (gram) for (a) Mavromichalis *et al*. (2001) piglets 5-10 kg BW (♦), (b) Wiltafsky (2009) piglets 8-25 kg BW (×), (c) Mavromichalis *et al*. (2001) piglets 10-20 kg BW (■), (d) Nemecheck *et al*. (2010) piglets 6.8-11.36 kg BW (▲), (e) Theil *et al*. (2004) piglets 8-20 kg BW (•), (f) Barea *et al*. (2009) piglets 12-25 kg BW (●), (g) Gaines *et al*. (2011) piglets 13-27 kg BW (–), (h) Gaines *et al*. (2011) piglets 21-32 kg BW (+).

CHAPTER 3

FEED INTAKE, GROWTH AND CARCASS COMPOSITION OF WEANED PIGLETS RECEIVING VARYING LEVELS OF VALINE AND LEUCINE IN THEIR DIETS

3.1. INTRODUCTION

Formulation techniques of today focus on supplying diets that limit excess supply of amino acids (AAs). This technique attempts to supply an available AA balance that meets the requirement at metabolic level as close as possible. For this to occur, the requirement for each essential amino acid (EAA) must be known. Availability of synthetic AAs allows nutritionists to formulate feeds whose protein content consists of an AA pattern resembling that of the protein being synthesised in the body. Numerous studies have been conducted to determine the requirements for first four limiting AAs such as lysine, methionine, tryptophan and threonine. According to Figueroa *et al.* (2003), valine is the next limiting AA that limits growth. Defining the optimal level of valine required in pig feeds will contribute to supplying a feed AA profile that more closely resembles that of an ideal protein.

Only few attempts have been made to determine valine responses in weaned piglets (Barea *et al.*, 2009; Wiltafsky, 2009; Nemecheck *et al.*, 2010; Gaines *et al.*, 2011). Most of these experiments have aimed to determine the optimal valine-to-lysine requirement ratio and most have used the formulation technique in which a basal feed is created that is deficient in the AA under test. The basal feed is then gradually supplemented with the test AA to see at which supplementation level growth rate is optimal. This technique is subjected to criticism mainly because the continuous addition of the limiting AA to the basal diet creates a series of diets in which the AA balance is not the same. Differing AA balances have been shown to substantially affect results. Secondly, at high supplementation levels the AA being tested may no longer be first-limiting and there might be an additional response if new first-limiting AAs were supplemented to the diet (Fisher & Morris, 1970; Gous & Morris, 1985). Fisher & Morris (1970) suggested the use of a summit dilution technique, which is a formulation technique that can overcome these disadvantages to a large extent.

Numerous authors have shown that oversupplementation of one branched chain amino acid (BCAA) can affect growth rate (Edmonds & Baker, 1987; Gatnau *et al*., 1995; Langer & Fuller, 2000; Peganova & Eder, 2003). Excessive intake of leucine is known to create a deficiency in valine and could therefore increase the requirement of valine in diets containing excessive amounts of leucine. Supplementation of valine to a diet could alleviate negative effects caused by high leucine intakes.

The objective of this study was to use a summit dilution technique to measure the response of weaned piglets to a range of diets varying in valine content, and to measure any interaction that might occur with supplementation of excess leucine to diets. These responses would make it possible to determine the dietary valine concentration at different leucine levels that would optimise growth rate and carcass leanness.

3.2. MATERIALS AND METHODS

The study was approved by the Animal Use and Care Committee of the University of Pretoria (EC042-10). The experiment was conducted at Ukulinga Research Farm of the University of KwaZulu-Natal, Pietermaritzburg, South Africa.

3.2.1 Animals and experimental design

The 75 Large White x Landrace entire male pigs used in the trial were purchased from a commercial farm near the experimental site. Pigs were weaned at 28 d of age and received a commercial weaner diet for two weeks. After they were moved to the experimental site, the pigs were continued on previously used commercial weaner feed for two days before being ear tagged and weighed. Piglets were housed individually and placed randomly in pens. Each piglet was randomly allocated to one of 18 treatments using a randomised blocks design. Because the environmental conditions along the length of the building were not identical, all treatments were represented in each of four blocks, these blocks consisting of 18 pens (nine on either side of the passage). The mean starting weight for all piglets was 13.46 ± 1.18 (mean ± SD) kg (min. 11.3, max. 16.7 kg), when they were placed on the experimental treatments. Three piglets were randomly selected to be slaughtered at the start of the trial to determine initial body composition. Each of the remaining 72 piglets was placed on 1 of 18 treatments. Pigs and feed were weighed on days 0, 7, 14 and 18 to calculate growth rate and feed intake.

After 18 days, remaining feed (orts) and piglets were weighed. All pigs were then slaughtered for carcass lipid, water, ash and protein analysis.

Each pen was equipped with a nipple drinker and a plastic self-feeder bin (Big Dutchman). Animals received feed and water *ad libitum.* The crate floors were suspended approximately 30 cm from the ground to allow faeces, urine and waste water to pass through the plastic slatted floor to the ground. Feed that was wasted by piglets from the feeders was collected by means of a tray underneath the feeder, which was emptied into a bucket daily and weighed weekly. Each crate was 1 m in length and height and 60 cm wide. Figure 3.1 represents the layout of the individual cages.

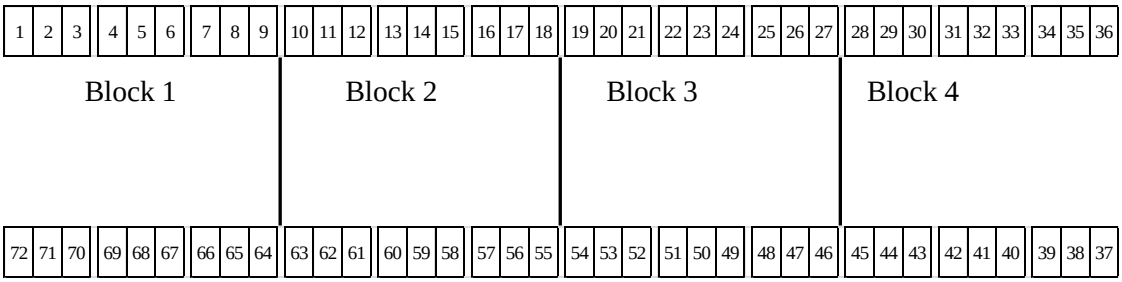


Figure 3.1 Diagrammatic representation of the layout of the individual cages

3.2.2 Diets and feeding

A dose response trial was designed to find the optimal dose that would maximise efficiency of valine utilisation for protein growth (g valine/ kg protein gain) at different levels of inclusion of leucine. The dilution technique was used to acquire the required levels of AAs in test diets (Fisher & Morris, 1970). One basal feed was formulated, mixed and divided into three summit feeds (S). One dilution mix (Dil) was formulated to contain the same concentration of all nutrients in the summit feeds, but without CP. Summit mix 1 was not supplemented with leucine (L1), summit mix 2 was supplemented with 20 g leucine/ kg feed (L2) and summit mix 3 was supplemented with 40 g leucine/ kg feed (L3) (Table 3.1). The objective was to create one series of valine concentrations at required leucine levels and two series of valine concentrations at leucine levels above requirement. Therefore, the second and third series (L2 & L3) had a moderately and severely imbalanced AA pattern, respectively. Each of three summit feeds was blended in suitable proportions with the dilution mix to produce a series of five feeds (Treatments 1 to 5) (Table 3.2). Summit feeds were blended as follows: (T1: 1.0 S;

T2: 0.85 S + 0.15Dil; T3: 0.70 S + 0.30 Dil; T4: 0.55 S + 0.45 Dil; T5: 0.40 S + 0.60 Dil). T6 was the same as T5 except that 1.8g crystalline valine per kg feed was added to T6. The objective of such an addition was to prove that valine was the first limiting AA if a significant response in growth would occur in pigs receiving T6. This created a series of six levels of valine (first limiting) at adequate, moderately excessive and excessive levels of leucine (T1 containing the highest and T5 the lowest valine concentration).

Table 3.1 The ingredient composition (g/kg fresh weight) of the summit and dilution mixes

Ingredient	Summit 1	Summit 2	Summit 3	Dilution
Yellow maize	202	202	202	
Maize gluten (600 g protein/kg)	44.7	44.7	44.7	
Wheat bran	157	157	157	
Soybean full fat	165	165	165	
Soybean (460 g protein/kg)	331	331	331	
L-Lysine HCl	12.4	12.4	12.4	
DL methionine	5.23	5.23	5.23	
L-Threonine	4.51	4.51	4.51	
L-Leucine	0	20	40	
Tryptophan	0.41	0.41	0.41	
Vit + Min premix	1.53	1.53	1.53	1.53
Sucrose				385
Starch				381
Filler[a]	40	20	0	160
Limestone	18.1	18.1	18.1	18.3
Salt				3.62
Monocalcium phosphate	12.2	12.2	12.2	20.0
Sodium bicarbonate	6.87	6.87	6.87	1.52
Oil- sunflower				28.6

[a] Filler was composed of sunflower husks (52.38%) and sand (47.62%)

The dilution percentages of summit diets and expected valine and leucine concentrations of treatments are given in Table 3.2.

Table 3.2 Dilution percentages of summit diets and expected digestible valine and leucine concentrations (g/kg)

Leucine	Valine	Dilution (%)	Valine	Leucine
L1	V1	0	11.9	23.8
	V2	15	10.1	20.2
	V3	30	8.3	16.6
	V4	45	6.6	13.1
	V5	60	4.8	9.5
	V6[a]	60 + supplement	6.6	9.5
L2	V1	0	11.9	45.1
	V2	15	10.1	38.3
	V3	30	8.3	31.6
	V4	45	6.6	24.8
	V5	60	4.8	18.0
	V6[a]	60 + supplement	6.6	18.0
L3	V1	0	11.9	67.1
	V2	15	10.1	57.0
	V3	30	8.3	47.0
	V4	45	6.6	36.9
	V5	60	4.8	26.8
	V6[a]	60 + supplement	6.6	26.8

[a] T6 was supplemented with synthetic valine at 1.8g/kg feed.

The only differences between the three summit diets were that summit mix 1 contained 40g filler/kg, summit mix 2 contained 20g filler/kg and 20g leucine/kg and summit mix 3 contained 40g leucine/kg. The analysed chemical composition of AAs, CP, neutral detergent fibre (NDF) (g/kg) and calculated digestible energy (DE) of the summit and dilution feeds are given in Table 3.3. The higher CP content of summit 2 and summit 3 are due to supplemented leucine which is also the reason for higher DE values for summit 2 and summit 3.

One sample of each summit mix and dilution mix was collected and submitted to the laboratory for proximate analysis consistent with AOAC (2000) methods. The total AA composition was analysed using the following equipment: The auto-sampler injects the

required amount into the analytical stream. Spark Midas, P.O. Box 388, 7800 AJ Emmen, The Netherlands. The Knauer Smartline Pump 1000 and Knauer Smartline Manager 5000 control the ratio of the two reagents used in the analytical flow stream. Knauer, Advanced Scientific Instruments, Hegauer Weg 38, D-14163, Berlin, Germany (www.knauer.net). The Pickering module is the standard detection system featuring the classical Moore-Stein ninhydrin reagent. This requires heating the combined column effluent and reagent mixture for a period, prior to survey by the detector. The Dionex PDA-3000 Photodiode Array Detector is an optical detector capable of measuring the absorbance spectrum from 190 to 800nm. Dionex Corporation, 1228 Titan Way, P.O. Box 3603, Sunnyvale, CA94088-3603. doc.065130-01 designed, developed and manufactured under an NSAI registered ISO Quality System (www.dionex.com). A Deuterium lamp optimises the UV range (190 to 380nm) and a Tungsten lamp optimises the visible range (380 to 800nm). Chromeleon is a modern chromatography management system that allows you to control and monitor all connected chromatography instruments. It enables quantitative and qualitative evaluation of the data using the Photodiode Array Detector. With photodiode array detectors, 3-D data provides spectral information that can be used for peak purity analysis and compound identification using spectral library matching. The digestible energy (DE) was calculated using the following equation: DE = 3.77-{[0.019 x (NDF % x 10)] + [0.758 x GE (MJ/kg)}.

Table 3.3 Analysed chemical composition of protein amino acid composition, crude protein (CP), digestible energy (DE) and neutral detergent fibre (NDF) (g/kg) of the summit and dilution feeds

	Feeds							
	Summit 1		Summit 2		Summit 3		Diluent	
Nutrient	Total	Dig.[#]	Total	Dig.	Total	Dig.	Total	Dig.
CP	303	-	318	-	330	-	6.1	-
NDF	153	-	135	-	114	-	50.6	-
DE (MJ/kg)	13.6	-	14.2	-	14.9	-	13.8	-
Cysteine	4.3	3.9	4.3	3.8	4.3	3.7	0.6	*
Methionine	11.5	10.5	12.0	10.8	11.3	10.1	0.2	*
Aspartic Acid	29.1	26.5	30.8	26.4	27.9	22.6	0.5	*
Threonine	14.2	13.2	15.1	13.1	14.6	12.3	0.3	*
Serine	12.8	12.1	13.3	11.5	12.9	10.8	0.2	*
Glutamic Acid	49.7	46.2	52.8	47.6	48.5	42.4	0.9	*
Proline	13.9	12.7	15.4	13.6	15.2	12.5	0.2	*
Glycine	11.7		12.3		10.9		0.4	*
Alanine	14.3	12.5	15.3	13.0	14.3	11.8	0.3	*
Valine	14.0	12.4	14.6	12.4	12.5	11.0	0.3	*
Isoleucine	12.5	11.1	12.6	10.8	10.8	8.7	0.2	*
Leucine	26.1	23.8	48.4	45.1	70.8	67.1	0.5	*
Tyrosine	8.7	7.6	8.8	7.6	8.5	6.8	0.0	*
Phenylalanine	14.0	12.5	14.5	12.6	13.3	11.1	0.2	*
Lysine	21.3	19.6	22.9	20.7	21.5	19.0	0.2	*
Histidine	7.1	6.2	6.9	6.0	6.2	5.1	0.1	*
Arginine	17.4	16.1	17.9	16.1	16.2	13.8	0.2	*

*Protein too low to calculate digestible amino acids
[#]SID amino acid concentration of the feeds

3.2.3 Slaughter procedure

Pigs were euthanised by intra-cardiac injection of sodium pentobarbital (1ml/kg body weight). After euthanasia, pigs were cut open to remove and strip the gastrointestinal tract (GIT) from its contents. The empty body and GIT were stored in plastic bags and frozen at -20℃. Each frozen carcass with viscera and GIT was minced and homogenised in a mincer. One sample was collected from each homogenate and placed in a 500g container. All samples were submitted to the laboratory for proximate analysis consistent with AOAC (2000) methods, with the exception for lipid, which was calculated from gross energy (GE) and true or crude protein content of the carcass according to the method as stated by Ferguson *et al.* (2000) (Equation 3.1).

Lipid = (2.410 x GE) – (0.5898 x protein) [g/kg DM] ***(3.1)***

GE is the carcass gross energy expressed in MJ/kg DM and protein is the carcass protein content, g/kg DM.

The dry matter (DM) content of each sample was determined by freeze-drying each sample for 48 hours. Second moisture content was determined by placing each sample in a 90^0C oven for 24 hours. The protein content was analysed on the FP2000. Energy is determined by using a LECO AC500 automatic bomb calorimeter. LECO Corporation, 3000 Lakeview Avenue, St Joseph MI 49085-2396, USA.

3.2.4 Statistical analysis

Feed intakes and body weights of all pigs from each treatment were pooled to determine the average feed intake and body weight for each of the 18 treatments. Valine concentration of each treatment was calculated from the analysed valine concentration of the respective summit feed and its proportion in the final mix. Valine intake of each pig was calculated from average daily feed intake and the valine concentration of the feed. Mean initial carcass protein content was calculated from carcass protein contents of the three pigs that were slaughtered at the start of the trial. Carcass protein weight of each pig was determined at the start and the end of the trial by using the mean initial and final carcass protein contents multiplied by initial and final body weights, respectively. Mean daily protein gain of each pig was calculated as the difference between the initial and final carcass protein weights divided by the length of the trial period. Carcass lipid contents for each treatment were calculated using mean carcass contents of all pig samples and mean body weights of all pigs on that treatment. Treatment means were obtained by using the general analysis of variance in Genstat.

The relationship between valine intake and carcass protein gain was determined using a statistical program that fits the Reading Model (Fisher *et al*. 1973) to the data for each leucine series. This program produces two coefficients that describe this relationship, namely, the amount of valine (mg) required per kg body weight for maintenance, and the amount of valine (mg) required per g of protein gain.

3.3. RESULTS AND DISCUSSION

All studies involved in determining the valine requirements of weaned piglets have used the graded supplementation technique. The basis of this method is to produce a diet severely limiting in valine and then to add successive doses of synthetic valine thereby obtaining a response curve from which the optimum level of valine supplementation that yields the best growth performance is determined. The resultant valine requirement is expressed either as standardised ileal digestible (SID) valine-to-lysine ratio (Chung & Baker, 1992; Barea *et al*., 2009), percentage SID valine in the diet (Gaines *et al*., 2011) or SID Val/MJ of ME (Mavromichalis *et al*., 2001). When the requirement is expressed as the optimal valine-to-lysine ratio, lysine has to be the second limiting AA. This method has been criticised mainly because the successive additions of valine change the AA balance at each dose, and it is difficult to compile a basal diet which is adequately deficient in valine yet sufficient in all other AAs, making it possible to examine only a narrow range of valine supplementation levels. Furthermore, at high valine supplementation levels, valine may no longer be first limiting and there might be an additional response if new first limiting AAs were supplemented to the diet (Fisher & Morris, 1970; Gous & Morris, 1985).

Due to the limitations of the graded supplementation technique, the summit dilution method was used in this study. With this method, the AA under test is always the first-limiting AA and ensures that the AA balance remains constant throughout the dilution series. This ensures that the response to one EAA is measured without the presence of other compounding effects. Results obtained in this study may therefore differ slightly from other studies. In addition, pig trials are usually conducted between two body weights whereas broiler trials are conducted over a fixed period of time. In this study, an 18 d trial period was used, which could lead to slightly different responses in feed intake when compared with other pig studies where a fixed final weight had to be achieved in each treatment.

This study investigated the response of weaned piglets to varying levels of valine and leucine in weaner pig feeds. The response in average daily gain (ADG), average daily protein gain (ADPG), average daily feed intake (ADFI), average daily valine intake (ADVI) and feed conversion efficiency (FCE) are shown in Table 3.4. Responses were seen in ADG, ADPG, ADFI, ADVI and FCE on valine treatment 6 (V6) at all three leucine levels when these were compared with the responses to treatment 5, which had essentially the same nutrient content as

treatment 6, but with an additional dose of valine. That the pigs responded to the additional dose of valine proved that valine was the first limiting AA in all diets in the series, given that the dilution feed was devoid of protein.

3.3.1 Feed intake changes

Mavromichalis *et al*. (2001), Barea *et al*. (2009) and Gaines *et al*. (2011) found that feed intake (FI) was reduced as the dietary valine:leucine decreased due to a valine deficiency. Lewis & Nishimura (1995) found that ADFI was unaffected by feed valine percentage. However, in the present trial, ADFI increased as dietary valine concentration decreased at normal leucine levels (Table 3.4). These findings suggest that when the feed is balanced and the dietary valine concentration decreases, pigs increase their FI to satisfy their valine requirement for growth and maintenance, as suggested by the food intake theory of Emmans (1981). A moderate excess of leucine in the diet resulted in a slightly imbalanced feed. Such an imbalanced feed caused an even greater increase in ADFI with decreasing valine concentration, but ADFI suddenly decreased when valine concentration dropped below 8.3 g/kg feed. The increase in ADFI, although the feed was slightly imbalanced, suggests that even when an excess leucine causes a deficiency in valine, the deficiency was overcome through an increased FI. When feed valine content decreased below 8.3 g/kg, the effect of excess leucine caused FI to fall sharply. This reduction in FI at a low valine concentration is probably a combined effect of an imbalance between that nutrient and energy and a reduced growth rate (Burnham *et al*., 1992). The same effect was seen when pigs were fed the severely imbalanced feed. However, pigs decreased FI when dietary valine concentration decreased and could not compensate for a reduction in valine concentration by increasing FI sufficiently.

There is substantial evidence that verifies the existence of antagonistic effects of excess leucine on animal performance (D'Mello & Lewis, 1970; Gatnau *et al*., 1995; Baker, 2005). Burnham *et al.* (1992) found that an imbalanced feed caused by excess leucine in feed results in a depressed FI, which is consistent with the present results. The responses in ADFI that have been seen when dietary valine content was decreased at adequate, moderately excessive and excessive levels of leucine, indicated that pigs attempted to compensate for lower dietary valine content by increasing FI. However, the more imbalanced the feed became, the more difficult it became to compensate for the decreasing dietary valine content by increasing FI. At the lowest valine levels, all other AAs were also at their lowest levels, but still adequate for

growth and maintenance. This means that a valine was deficient in all diets and a deficiency of an AA other than valine could not have caused the effects seen on ADFI.

The effect of excess leucine content was greatest at the lowest valine intake. FI regulation is the homeostatic response that regulates the AA concentration in plasma and tissues by reducing the influx of AAs (Harper, 1974). The three BCAAs are structurally similar, share common systems for transport through cell membranes and use the same enzymes for degradation. An excessive intake of one BCAA may result in an increase in the concentration of that particular BCAA in blood, which may lead to depletion of the other BCAAs in the brain, causing secondary anorexia. This is one major factor that causes a drop in FI due to an imbalanced feed (Peganova & Eder, 2003). A second factor is that high leucine intake stimulates activity of branched-chain α-keto acid (BCKA) dehydrogenase which is the enzyme involved in the breakdown of all three BCAAs (Harris *et al*., 2001; Baker, 2005). The resultant increased oxidation of valine causes a deficiency in this AA, which results in a performance depressing effect (Smith & Austic, 1978). Thirdly, high plasma leucine levels indicate to the organism that protein intake was sufficient and that intake of feed can be ceased (Wiltafsky, 2009).

Table 3.4 Mean daily gain (ADG), protein gain (ADPG), feed intake (ADFI), valine intake (ADVI) and feed conversion efficiency (FCE) (g BW gain/kg feed) of pigs over the 18 d trial period

Leucine	Valine treatment	Feed valine content (g/kg)	Valine intake (g/day)	ADG (g/d)	ADFI (g/d)	ADPG (g/d)	FCE
L1	V1	11.9	9.08	514	763	100	680
	V2	10.1	9.35	672	923	127	731
	V3	8.3	7.76	615	930	104	663
	V4	6.6	6.77	610	1033	103	590
	V5	4.8	4.98	529	1045	77	506
	V6[c]	6.6	6.90	588	1051	97	553
	Mean		7.47	588	957	101	620
L2[a]	V1	11.9	9.63	629	808	117	783
	V2	10.1	9.26	637	914	120	701
	V3	8.3	8.50	675	1019	112	674
	V4	6.6	4.33	349	661	58	525
	V5	4.8	3.08	239	646	38	363
	V6[c]	6.6	7.74	643	1179	105	545
	Mean		7.09	529	871	92	598
L3[b]	V1	11.9	9.81	569	824	108	701
	V2	10.1	7.97	528	787	96	668
	V3	8.3	6.05	437	726	76	595
	V4	6.6	3.68	260	562	49	460
	V5	4.8	2.61	168	548	30	301
	V6[c]	6.6	6.18	519	941	75	548
	Mean		6.05	414	731	72	545
	RMS[d]		1.45	10.3	22.27	351	4664

[a] Treatments were supplemented 20 g synthetic leucine / kg feed.
[b] Treatments were supplemented 40 g synthetic leucine / kg feed.
[c] The composition of V5 and V6 was the same except that V6 was supplemented with 1.8g synthetic valine / kg feed.
[d] Residual mean square.

3.3.2 Growth and efficiency

As shown in Table 3.4, FI increased when feed valine concentration dropped at a normal leucine level and this initially caused an increase in ADG, but as feed valine concentration dropped below 10.37 g/kg feed, an increase in FI did not compensate for the lower valine concentration in the feed. This resulted in a lower ADG and consequently in a sharp reduction in feed conversion efficiency (FCE) (g BW gain/kg feed intake). When the feed was slightly

imbalanced, a lower feed valine content could be compensated through increased FI, but at the cost of FCE. However, when valine content fell below 8.54 g/kg feed, FI and ADG decreased severely. When the feed was severely imbalanced there was a substantial reduction in FI, ADG and FCE. These results suggest that leucine given in excessive amounts causes increased oxidation of valine and decreased performance even at the highest dietary valine concentration. It also appears that the negative impact on ADG of animals caused by excess leucine intake is more severe at lower feed valine concentrations. At normal and moderate excessive leucine levels, ADG of pigs was lower for the highest than the second highest dietary valine concentration. A possible reason for this is that at the highest valine and protein level, the ratio of energy:protein was too low and the energy concentration in the feed was not high enough for the animals to utilise all the protein (Kyriazakis and Emmans, 1992 a and b).

3.3.3 Body composition and efficiency

Baker (2005) fed pigs a diet containing about six times the required level of leucine and found that this level of surplus leucine caused pigs to deposit more lipid in muscle compared to the control level. However, in this study, carcass composition was not affected by feeding excess leucine to pigs (Table 3.5). The valine concentration, however, had a tendency to effect carcass composition. Carcass protein content decreased as dietary valine concentration decreased. This effect was seen at all three leucine levels. Figure 3.2 shows that as valine concentration of the feed decreases at all three leucine levels, FCE decreases as pigs increase FI to compensate for a lower feed valine content. An increased FI leads to excess energy taken in by the pig which is consequently stored as carcass lipid. Landgraf *et al*. (2006) found that with an increased empty body lipid percentage, the drop in percentage body water was almost inversely proportional (slightly higher increase in lipid percentage), which is consistent with the results of this study. As the dietary valine concentration decreased, carcass lipid percentage increased and carcass water percentage decreased. It is clear that pigs attempted to compensate for a valine deficiency in the diet by increasing FI, thereby consuming more energy than required and this energy was stored as fat. However, it seems as if this is not the only factor that causes an increase in carcass lipid percentage because at excessive leucine intakes, even though FI drops, carcass lipid content is increased.

Table 3.5 Mean final body composition of pigs (g/kg)

Leucine	Valine treatment	Feed valine content	Valine intake (g/day)	Water	Protein	Lipid	Ash
L1	V1	11.9	9.08	731	166	118	28
	V2	10.1	9.35	718	166	131	27
	V3	8.3	7.76	724	156	129	27
	V4	6.6	6.77	715	155	135	28
	V5	4.8	4.98	695	146	157	29
	V6[a]	6.6	6.90	689	153	155	28
	Mean		7.47	712	157	137	28
L2[c]	V1	11.9	9.63	743	164	121	25
	V2	10.1	9.26	732	166	116	26
	V3	8.3	8.50	717	155	139	27
	V4	6.6	4.33	707	152	145	31
	V5	4.8	3.08	709	148	150	27
	V6	6.6	7.74	687	153	160	30
	Mean		7.09	716	156	138	28
L3[d]	V1	11.9	9.81	726	165	126	27
	V2	10.1	7.97	725	161	126	25
	V3	8.3	6.05	718	157	130	28
	V4	6.6	3.68	719	157	135	27
	V5	4.8	2.61	702	151	151	28
	V6	6.6	6.18	689	145	158	28
	Mean		6.05	713	156	138	27
	RMS[b]		1.45	27.4	0.54	2.23	0.087

[a] The composition of V5 and V6 was the same except that V6 was supplemented with 1.8g synthetic valine / kg feed.
[b] Residual mean square.
[c] Treatments were supplemented 20 g synthetic leucine / kg feed.
[d] Treatments were supplemented 40 g synthetic leucine / kg feed.

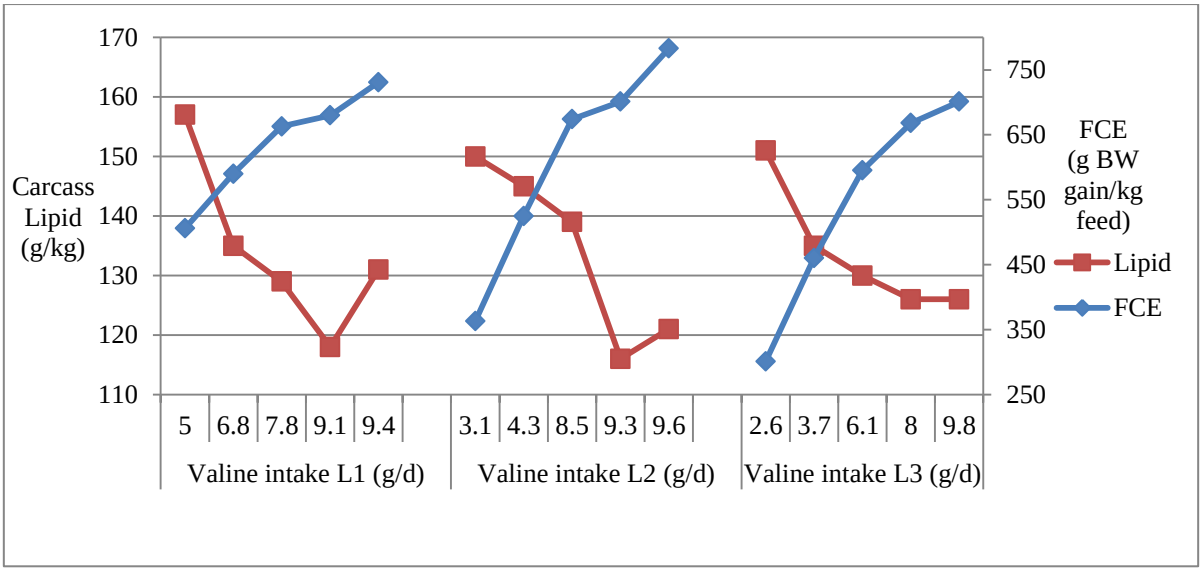


Figure 3.2: Relationships between valine intake (g/d), for balanced (L1), moderately imbalanced (L2) and severely imbalanced treatments (L3), and final carcass lipid content (g/kg) and FCE (g BW gain/ kg feed intake)

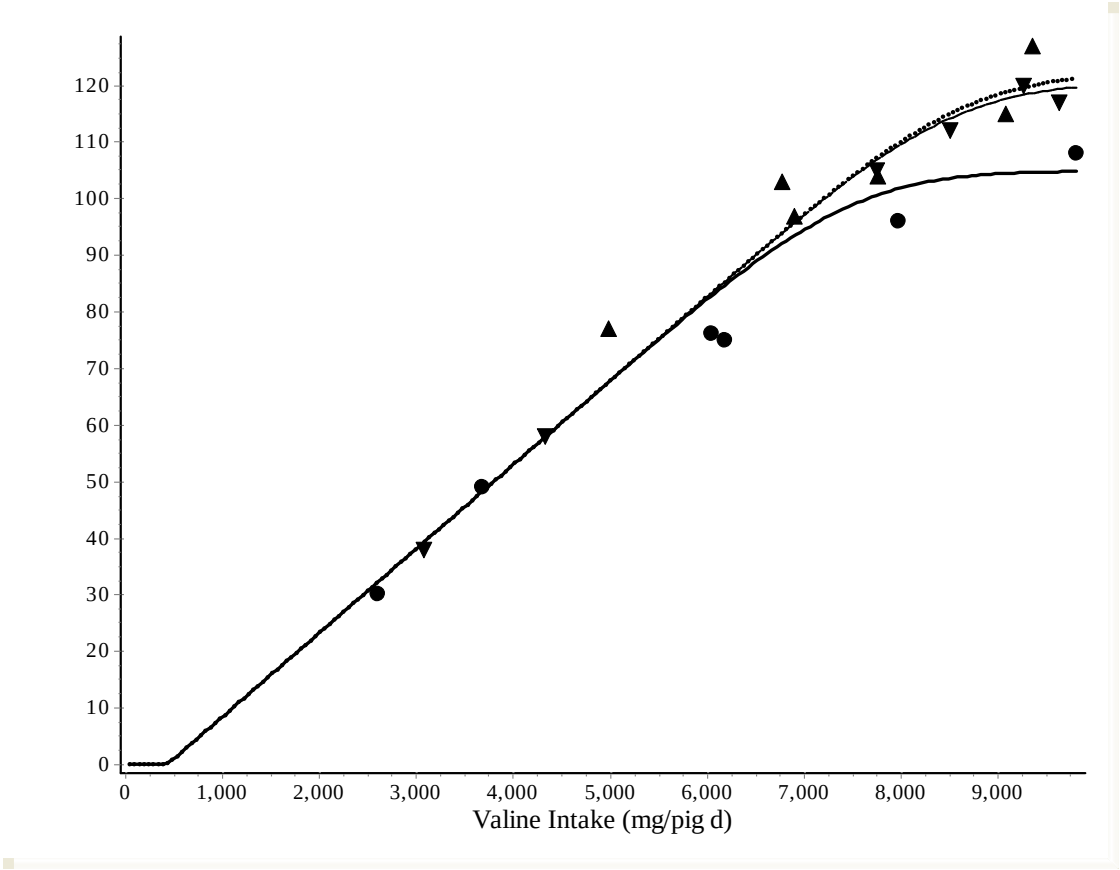


Figure 3.3: The response in protein gain in weaner pigs to valine intake for the balanced (▲), moderately imbalanced (▼) and severely imbalanced (●) treatments. Imbalances were achieved by addition of crystalline leucine to the diets

When the Reading Model was fitted to the response data for each leucine series (Fig. 3.3), the amount of valine required per kg of body weight for maintenance was found to be 29.4 mg and 67.3 mg per g of protein gain. These coefficients were unaffected by the amount of leucine in the feed, as illustrated in Fig. 3.2. The valine content of pig protein, according to the ARC (1981) is 49 g/kg protein. Therefore, the efficiency of valine utilisation for protein growth is 49/67.3 = 73%. A SID valine intake of 9000 mg/d or more will not result in any further increase in protein growth, and if the feed is severely imbalanced, a SID valine intake of 8000 mg/day or more will not result in a further increase in protein growth.

3.4. CONCLUSION

This experiment is distinctive in that it measured the response of weaned piglets to varying levels of dietary valine and leucine making use of the diet dilution technique. Furthermore, the amount of valine that would maximise protein growth was determined, as were the coefficients relating the amount of valine required per kg body weight for maintenance and the amount required per mg for body protein growth. The results in this paper indicate that, over the range of valine concentrations used in the study, leucine did interact with valine and that increasing the leucine to valine ratio resulted in poorer growth, but only at low valine concentrations. This depression of growth could be explained by the drop in feed intake that resulted from feeding such an imbalanced diet. The depression of feed intake was not obvious at high valine levels, but the diet containing the lowest valine content, which was most severely imbalanced, caused feed intake and growth to be severely depressed. However, valine supplementation to such an imbalanced diet was found to reverse such a depression of feed intake and growth. The experiment confirms that excess leucine levels, which could often occur in commercial feeds containing DDGS and maize gluten, may cause an imbalanced AA pattern which affects growth and feed intake, especially when valine is given in low amounts. Because isoleucine is also implicated in the possible imbalance between leucine and valine, it would be useful to evaluate the response of a leucine imbalanced feed to decreasing levels of isoleucine when valine is given in required amounts.

The coefficients of response to valine were determined to be 29.4 mg Val/ kg BW and 67.3 mg Val/ g protein gain. The efficiency of utilisation of valine was found to be 73 % and that an intake of around 9 g valine per day will yield maximal protein growth in pigs of the genotype used, and over the period of growth used in this trial. The optimum economic intake of valine would depend on the marginal cost of valine and the marginal revenue for pig protein, which is academic under these circumstances given that pigs would not normally be sold at the weights achieved in this trial.

CHAPTER 4

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