1 General Introduction: The Concept of Standardised Ileal Amino Acid Digestibilities: Principles and Application in Feed Ingredients for Piglets

1.1 Summary

In this review, the terminology that is used to describe ileal amino acid (AA) digestibilities in piglet feed ingredients is defined. If one accepts that the determination of AA digestibilities should be based on the ileal analysis method, one should consider that ileal digesta contains variable amounts of endogenous crude protein (CP), which originates mainly from digestive secretions, sloughed-off epithelial cells and mucins. The ileal endogenous CP and AA losses are separated into basal ileal endogenous CP and AA losses ($IAAL_B$), which are not influenced by the feed ingredient composition, and specific ileal endogenous CP and AA losses ($IAAL_S$), which are induced by feed ingredient characteristics such as level and type of fibre and anti-nutritional factors (ANF). Depending how ileal endogenous CP and AA losses are considered in the measurement of CP and AA digestibilities, digestibility values are expressed as apparent (AID), standardised (SID), or true (TID) ileal digestibilities of CP and AA. The main concern associated with the use of AID values in diet formulation for pigs is that they are not additive in mixtures of feed ingredients. Consequently, the concept of standardised ileal CP and AA digestibilities was introduced by correcting AID values for basal ileal endogenous CP and AA losses ($IAAL_B$). The correction for both, $IAAL_B$ and $IAAL_S$ yields in TID values, however, routine procedures to measure $IAAL_S$ are not yet available. In principle, SID values should be preferred, because they represent the fundamental properties of the feed ingredient. There exist only few reports on SID of CP and AA in feedstuffs frequently used in piglet nutrition. These include soybeans (SB), soybean meal (SBM), soy proteins (SP), soy protein concentrate (SPC), soy protein isolate (SPI), corn gluten (CG), wheat gluten (WG), pea protein (PeaP), potato protein (PotP), fish meal (FM) and whey proteins (WP), but the results obtained are inconsistent. Differences in SID values within feed ingredients may, at least in part, be attributed to different processing conditions or inherent differences of the assay feed ingredients. Moreover, there is some evidence that the determination of SID values and $IAAL_B$ in piglets may be confounded by the dietary CP level of the assay diet, age and (or) body weight (BW), the level of feed intake or the methodological approach used to determine $IAAL_B$.

1.2 Introduction

Feed costs represent at least 55 to 65% of the variable costs in swine production, and play a major role in determining the profitability of a swine enterprise, especially in view of recent increases in feed costs (Yacentiuk, 2001; FAO, 2008). Diets which accurately match the piglet's protein requirement rely, firstly, on the exact knowledge of the animal's requirement for indispensable amino acids (AA) and, secondly, on a precise description of feed ingredients in terms of their capacity to supply these AA for maintenance and growth (Williams, 1995).

The protein quality among feed ingredients varies considerably (Knabe et al., 1989; Yin et al., 1993), and is governed by the AA composition and ratios of indispensable AA, as well as the susceptibility of the protein to be hydrolysed during digestion (Friedman, 1996). Feed processing is considered to be a major factor which may influence protein quality including digestibility and bioavailability of AA (Camire et al., 1990; Singh et al., 2007). Since measurements of AA availabilities are considered to be time-consuming, labor-intensive and expensive, the determination of AA digestibilities at the ileal level has proven to be a more suitable approach (Mosenthin et al., 2000). Moreover, there is a general agreement that the expression of standardised ileal digestibilities (SID) of crude protein (CP) and AA has advantage over apparent (AID) and true (TID) ileal CP and AA digestibilities, because SID represent the fundamental properties of the individual feed ingredient (Mosenthin and Rademacher, 2003). In this context, a careful assessment of SID of CP and AA is critical for evaluating the protein value of feed ingredients for piglets (Stein et al., 2007).

The SID of CP and AA can be obtained by correcting AID values for basal ileal endogenous CP and AA losses ($IAAL_B$) (Stein et al., 2007). As a result, measurements of SID, in contrast to AID, have proven to be nearly independent of the dietary CP and AA contents in assay diets fed either to grower-finisher pigs (Fan et al., 1995a) or piglets (Eklund et al., 2008b). Thus, SID values have been shown to be additive in mixed diets for grower-finisher pigs (Stein et al., 2005).

Estimates of SID have been generated for most feed ingredients in grower-finisher pigs (NRC, 1998; AmiPig, 2000; Rademacher et al., 2001; Pedersen and Boisen, 2002; CVB, 2003; GfE, 2008), whereas corresponding SID values for piglets hardly exist. Piglets

are assumed to have a limited capacity for CP digestion and AA absorption (Pluske et al., 1997; Hedemann and Jensen, 2004). In addition, factors such as body weight (BW) and (or) age (Caine et al., 1997a,b), and the level of feed intake (Moter and Stein, 2004; Diebold et al., 2005) may affect AID, SID and $IAAL_B$ in pigs. Therefore, it remains open, whether SID of CP and AA determined in feed ingredients for grower-finisher pigs can be used in diet formulation for piglets as well.

This review aims to summarize the principles of the concept of SID of CP and AA and its impact on protein evaluation in feed ingredients for piglets.

1.3 Expression of Crude Protein and Amino Acid Digestibilities

1.3.1 Total Tract Digestibilities

The digestion of AA in the large intestine is the result of microbial activity (Sauer and Ozimek, 1986; Mosenthin and Rademacher, 2003). The disappearance of AA from this part of the digestive tract would not be necessarily a problem if disappearance represented absorption of AA (Mosenthin and Rademacher, 2003). However, CP digestion through microbial activity does not contribute to maintenance or tissue accretion, because the absorbed end-products of microbial fermentation are ultimately excreted in the urine (Mosenthin and Sauer, 1992). Since AA are only absorbed proximal to the distal ileum, ileal digestibility measurements represent more accurate estimates of AA bioavailabilities than fecal AA digestibilities (Sauer and Ozimek, 1986).

1.3.2 Ileal Digestibilities

Apparent ileal digestibilities: The AID of CP and AA are defined as the net disappearance of ingested dietary CP and AA from the digestive tract proximal to the distal ileum (Tanksley et al., 1981; Sauer and Ozimek, 1986). AID values are calculated from the flow and composition of digesta at the distal ileum of pigs by determining the difference between the total ileal outflow of AA and the dietary intake according to the following equation:

$$AID\ (\%) = ((\text{CP or AA intake} - \text{ileal CP or AA recoveries}) / \text{CP or AA intake}) \times 100\%$$

The expression 'apparent' is used to emphasize that both, non-digested dietary CP and AA, and CP and AA of endogenous origin that are secreted into the gastrointestinal tract without being re-absorbed, contribute to the total ileal outflow (Stein et al., 2007). A primary concern with the use of AID values in diet formulation and feed evaluation is the lack of additivity of AID values in the formulation of complex diets (Stein et al., 2005).

Ileal endogenous losses: Ileal endogenous losses of AA represent AA that are present in endogenously synthesized CP (nitrogen×6.25) secreted into the intestinal lumen, and which have not been digested and re-absorbed before reaching the distal ileum (Tamminga et al., 1995; Hodgkinson and Moughan, 2000). Mucoproteins, sloughed cells, serum albumin, digestive enzymes, amides, and ingested hair as well as microbial CP contribute to endogenous CP losses (Souffrant, 1991; Nyachoti et al., 1997). The ileal endogenous losses of CP and AA can be divided into basal (non-specific) ileal endogenous CP and AA losses ($IAAL_B$) and specific ileal endogenous CP and AA losses ($IAAL_S$) (Jansman et al., 2002).

The $IAAL_B$ represent the minimum quantities of AA inevitably lost by the animal (Stein et al., 2007). These losses are considered to be related to the physical flow of feed dry matter (DM) through the digestive tract and (or) the animal's metabolic state, but are not influenced by diet composition (Stein et al., 2007). The $IAAL_B$ can be measured by feeding i) a CP-free diet, ii) a diet supplemented with a highly digestible CP source such as casein or wheat gluten, iii) a diet based on enzyme-hydrolysed casein plus ultra-filtration of the digesta, or iv) can be estimated by using different regression analysis methods (Jansman et al., 2002). For piglets, values for $IAAL_B$ are summarized in Table 1 (Eklund et al., 2008a,b).

The $IAAL_S$ are influenced by diet ingredients composition such as content and type of fibre and anti-nutritional factors (ANF) including trypsin inhibitors (TI), lectins or tannins (Jansman et al., 1995; Schulze et al., 1995). There are no routine procedures available to measure $IAAL_S$ in pigs. However, it is possible to calculate $IAAL_S$ by estimating total (basal plus specific) ileal endogenous CP and AA losses ($IAAL_T$) and then subtracting the $IAAL_B$ from $IAAL_T$. Procedures used to estimate $IAAL_T$ include the homoarginine method (Rutherfurd and Moughan, 1990) and isotope tracer dilution techniques (de Lange et al., 1990; Leterme et al., 1998).

Table 1: Basal ileal endogenous losses of crude protein and amino acids (g/kg DMI) and dietary threshold levels of crude protein and amino acids (g/kg DM) in piglets

Item	Basal ileal endogenous losses (g/kg DMI)		Threshold level[3] (g/kg DM)
	Regression analysis[1]	Casein[2]	
CP	9.3	16.3	176.3
Indispensable AA			
Arginine (Arg)	0.4	0.5	6.6
Histidine (His)	0.2	0.3	5.1
Isoleucine (Ile)	0.2	0.5	8.4
Leucine (Leu)	0.5	0.8	15.9
Lysine (Lys)	0.4	0.7	12.3
Methionine (Met)	0.1	0.2	4.8
Phenylalanine (Phe)	0.4	0.6	9.5
Threonine (Thr)	0.6	0.9	8.5
Tryptophan (Trp)	0.1	0.2	1.9
Valine (Val)	0.4	0.7	10.9
Dispensable AA			
Alanine (Ala)	0.5	0.7	5.4
Aspartic acid (Asp)	0.7	1.1	12.4
Glutamic acid (Glu)	0.9	1.8	35.0
Glycine (Gly)	0.8	0.8	3.9
Proline (Pro)	0.6	0.9	22.0
Serine (Ser)	0.6	0.8	9.3

[1] Eklund et al. 2008b, [2] Eklund et al. 2008a, [3] Eklund, 2007.

True ileal digestibilities: The TID, also referred to as real ileal digestibilities (de Lange et al., 1990; Souffrant, 1991), reflect the proportion of the dietary AA that disappears from the digestive tract proximal to the distal ileum (Stein et al., 2007). In this case, only the undigested dietary AA present in ileal outflow are related to the AA intake, whereas the proportion of $IAAL_T$ in ileal AA outflow is not accounted for (Furuya and Kaji, 1989; de Lange et al., 1990). The TID values are calculated the same way as AID values, except that the $IAAL_T$ are subtracted from the ileal recoveries of CP and AA according to the following equation:

$$TID\ (\%) = ((CP\ or\ AA\ intake - (ileal\ CP\ or\ AA\ recoveries - IAAL_T)) / CP\ or\ AA\ intake) \times 100\%$$

This approach allows the metabolic costs associated with synthesis and recycling of endogenous gut AA losses to be represented explicitly (Stein et al., 2007). There are only few reports on TID values of nitrogen and AA in skim milk powder, soybean meal, soy protein isolate and fish meal in weaned pigs (Caine et al., 1997b; Makkink et al., 1997). A major limitation to the use of TID values in piglet diet formulation is the lack of routine measurements for $IAAL_T$ which are additionally time consuming and expensive. Furthermore, TID values do not include the $IAAL_S$, and therefore require that those

unknown costs of protein digestion are included in the estimates of AA requirements. Consequently, the actual requirements for AA would depend on the ingested feed ingredients (Mosenthin, 2002).

Standardised ileal digestibilities: As an alternative to TID values, SID values have been introduced in diet formulation for pigs (Jondreville et al., 1995; Mosenthin et al., 2000). Using this approach, only $IAAL_B$ are subtracted from ileal outflow of CP and AA according to the following equation:

SID (%) = ((CP or AA intake-(ileal CP or AA recoveries-$IAAL_B$))
/CP or AA intake)×100%

If AID values have been already determined, these values can be transformed into corresponding SID values according to the following equation:

SID (%) = AID (%)+($IAAL_B$/CP or AA diet)×100%

The term 'true ileal digestibilities' describing the correction of AID values for $IAAL_B$ has been used to represent SID values (Furuya and Kaji, 1989; NRC, 1998). Moreover, different methods to obtain estimates of $IAAL_B$ have been applied. When a protein-free diet is fed, all protein containing compounds in ileal digesta are assumed to be of endogenous origin (Jansman et al., 2002; Pedersen and Boisen, 2002). The main criticism of this method is that animals are in negative nitrogen balance *per se*. The protein free diet may lack the stimulatory effect on endogenous gut secretions, which can lead to underestimation of $IAAL_B$. As an alternative to the protein-free diet method, diets containing protein sources with assumed 100% CP digestibility, such as casein, wheat gluten or crystalline AA have been used to determine $IAAL_B$ as reviewed by Jansman et al. (2002) and Pedersen and Boisen (2002). However, the validity of this method may be questioned until it is proven that these protein sources are indeed 100% digestible (Nyachoti et al., 1997). In various studies, $IAAL_B$ measured in different ways have been applied to calculate SID values in feed ingredients for piglets. For example, Walker et al. (1986) considered ileal CP and AA recoveries from piglets fed enzyme-hydrolysed casein to represent $IAAL_B$, whereas Yun et al. (2005b) and Yang et al. (2007) determined $IAAL_B$ in piglets fed protein-free diets.