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CONTENT

REVIEW OF AVAILABLE NUTRIENT DIGESTIBILITY VALUES IN WHITELEG SHRIMP

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KEY INFORMATION

- This article reviews the basic aspects of shrimp digestive physiology, the methodologies used to determine digestibility in shrimp, and the available nutrient digestibility values in whiteleg shrimp.

- Although the amount of nutrient digestible data available for shrimp is still limited, the number of studies reporting nutrient digestibility, including amino acid digestibility, has increased significantly during the last decade and are summarized in this article.

- The fit between digestible crude protein and digestible amino acid content is not consistent among ingredients, as reflected in the poor relationship seen when digestible crude protein is regressed against digestible lysine (R^2 of 0.63) or methionine (R^2 of 0.36) content of all of the 46 measurements for which these values are both available.

- Being a better criterion than total crude protein content, digestible protein content of an ingredient alone does not seem to be enough to accurately evaluate the quality of different ingredients.

- Further studies investigating the factors affecting nutrient, in particular amino acid digestibility of different ingredients in shrimp, would much contribute to the development of mathematical models estimating the digestible amino acid content in a more accurate way.

DEAR READER,
The first article is dealing with shrimp nutrition. Dr. Cláudia Silva reviews amino acid digestibility in shrimp.

The second article is about low protein diets. Based on the current knowledge, Dr. John Htoo investigates how much professional nutritionists today can reduce the dietary protein content without impairing performance.

Happy reading!

Vincent Hess

Vincent Hess

INTRODUCTION

Reduction of feed costs and safety margins while increasing the accuracy of predicting animals performance are major goals of a nutritionist. Knowledge of nutrient digestibility coefficients for individual ingredients and the requirement of digestible nutrients for a defined production target allows nutritionists to formulate diets that better match animals´ requirement. A major advantage of formulating diets in a digestible basis is that it ensures more predictable animal performance when changing feed recipes due to changes in availability of feedstuffs and feedstuffs prices. Moreover, protein and amino acid digestibility coefficients of ingredients are needed for more accurate, environmentally friendly, and economical feed formulations. Protein and amino acids are expensive nutrients in feed and thus oversupply of crude protein (CP) is the cause of nitrogen contamination of pond water and environmental pollution.

Data on amino acid digestibility coefficients is one of the most important factors in preparing adequate shrimp feeds and there is an increasing interest in defining feedstuff quality using as criterion the coefficients of amino acid digestibility. Shrimp feed is often formulated in terms of CP and amino acids content without considering the digestibility (nutrient content x digestibility coefficient) of these ingredients. This information is important, but is not sufficient for optimizing formulations. But, while still incomplete, significant information on the digestibility of nutrients, including proteins and amino acids from practical ingredients for pacific whiteleg shrimp (*Litopenaeus vannamei*), has been produced. The purpose of this article is to review and to produce tables with currently available data on nutrient digestibility for whiteleg shrimp, and thus to help making the process of least-cost formulation for shrimp more cost-effective and productive performance from diets more predictable. In addition, a short-review of the basic aspects of shrimp digestive physiology and of the methodologies used to determine digestibility in shrimp are provided.

BASIC ASPECTS OF THE DIGESTIVE ANATOMY AND PHYSIOLOGY OF SHRIMP

The major purpose of the digestive system is to provide the energy and nutrients required for maintenance, growth and reproduction requirements. Because physiological responses are essential to assess the animal performance at different environmental and feeding conditions, understanding digestion of nutrients by shrimp is an essential step towards achieving production targets.
Although *L. vannamei* are euryhaline and able to tolerate a wide range of salinity from 1 to 50 ppt (Pante, 1990), a salinity change does alter its growth performance and physiological responses (Fry, 1971; Kinne, 1971). The digestive tract of crustaceans is very small and thus, the mean gut passage time of feed is extremely short (60 to 80 minutes). Another important aspect to consider is the fact that *Penaeid* life history is marked by changes in morphology and behavior, with a shift from planktonic herbivory to omnivory in late protozoea and the adoption of a benthic existence as postlarvae. These ontogenetic events are accompanied by significant changes in metabolic rates and digestive enzyme activities (Laubier-Bonichon *et al.*, 1977; Lovett and Felder, 1989; Chu and Ovsianico-Koulikowsky, 1994; Lemos *et al.*, 1999). Furthermore, the digestive

system of shrimp is fundamentally different from those of fish or mammals, with stomach and intestine having completely different physiological functions as that known from vertebrates.

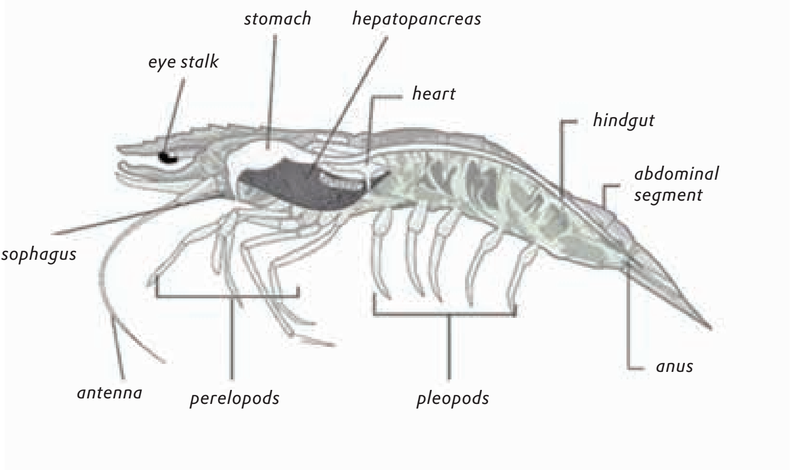
The anatomy and function of the digestive tract of shrimp have been described in detail elsewhere (Dall and Moriarty, 1983; Ceccaldi, 1997). Briefly, the digestive tract in shrimp is divided into three main compartments which are the foregut (mouth, oesophagus, stomach), the midgut (intestine and digestive gland) and the hindgut (rectum and anus) (Figure 1). The mouth of *Penaeid* crustaceans is surrounded by several appendages (maxillae, maxillulae, mandibles and maxillipeds) that are specialized for chemoreception, capture, manipulation and transport of feed to the mouth (Garm and Hoeg, 2001; New, 2002). Since the mouth itself mainly plays a role as a valve, the feed pellets are sorted and pre-minced outside the body using different mandibles, increasing probability of nutrients to be partly leached out of the pellets. Once in the mouth, feed pellets are swallowed and sent through the oesophagus where regular peristaltic contractions lead them to the cardiac pocket of the stomach. The lumen of the oesophagus is part of the external environment and its walls are covered with a thin layer of chitin, renewed with each moult (Guillaume and Ceccaldi, 1999).

The stomach is divided in two parts, the cardiac chamber and pyloric chamber and acts basically as a gastric mill with a subsequent filter unit. Hardly calcified and articulated structures called ossicles or teeth are present in the cardiac chamber, which are moved by the action of specific muscles that are connected at the external walls of the stomach bag. Folds, spines and bristles in the pyloric chamber allow only the finest feed particle to pass the pyloric chamber and enter the digestive gland (hepatopancreas). All feed components that enter the hepatopancreas must be ground to <1 µm and have to pass the filter unit of the pyloric chamber. Waste material is transported to the posterior intestine, where it is coated with a thin mucus layer and finally excreted by passing the hindgut. One particularity of the shrimp digestive physiology is the fact that their stomach has a neutral to slight alkaline pH, and it does not secrete enzymes. A further specificity is that the intestinal epithelium secretes mucus, which first coats the chyme leaving the stomach and then serves as a building block of the peritrophic membrane (i.e. chitin pellicula) of the faeces (Guillaume and Ceccaldi, 1999).

Nutrient digestion and absorption occur in the midgut and especially in the digestive gland (Dall and Moriarty, 1983), and it is unlikely to occur in the foregut or hindgut once they are both covered with a cuticle layer that prevents direct contact between cells and lumen. The overall uptake of nutrients seems fast as labelled food is observed in tissue one hour after feeding and completely absorbed after 4 to 6 hours (Dall *et al.*, 1990). The digestive gland or hepatopancreas accounts for 2 to 6 % of the total body mass, and is composed of 2 to 3 lobes further divided into ducts and tubules associated with connective tissue (Ceccaldi, 1997). This organ is connected to the digestive tract via principal ducts and functions in feed absorption, transport, secretion of digestive enzymes, and storage of lipids, glycogen, and a number of minerals. Four basic cell types have been described: E-(embryonic), F-(fibrillar), R-(resorptive), and B-(blister) cells, respectively (Sousa *et al.*, 2005). B-cells are large secretory cells which are the primary producers of digestive enzymes. The enzymes are continuously produced in the rough endoplasmatic reticulum as endocytotic vesicles which coalesce finally into one large vacuole within the B-cell. At the end of maturation, the vacuole is eliminated by holocrine excretion (Dall and Moriarty, 1983).

As pepsin is absent from proteolytic secretion in crustaceans (Galgani *et al.*, 1983; Vega- Villasante *et al.*, 1995), protein digestion is mostly done by serine endoproteases and exoproteases (Omondi, 2005). Trypsin-like enzyme is usually found and can represent up to one third of the soluble

Figure 1 Shrimp digestive system (source: FAO, 2001)



protein of digestive gland (Guillaume and Ceccaldi, 1999). Chymotrypsin-like activity is also found in the shrimp digestive gland (van Wormhoudt, 1974). In general, enzyme activities depend on several factors such as moult cycle (Fernández *et al.*, 1997), growth stages (Fang and Lee, 1992; Jones *et al.*, 1997) or dietary composition (van Wormhoudt *et al.*, 1980; Le Moullac *et al.*; 1996; Guzman *et al.*, 2001). For instance, a decrease of the amylase to total protease ratio was observed in *P. monodon* until postlarvae (PL) stage, remaining low at juvenile and adult stages, during which chymotrypsin activity is higher than that of trypsin (Fang and Lee, 1992). Also, van Wormhoudt *et al.* (1980) observed the highest protease activity when the shrimp *Palaemon serratus* was fed a diet containing 45 % protein, compared to other dietary protein levels. The relationships between protein level, protein source, size, and digestive protease enzyme activities of the marine shrimp, *Penaeus vannamei* Boone, were investigated during three 30-days growth experiments by Lee *et al.* (1984). The protein level influenced the enzyme activities in shrimp of all sizes while the protein source had a greater effect on the enzyme activities in small shrimp (< 10 g). This differing proteolytic response to protein level and source as a function of size, supports the formulation of specific diets for shrimp of different sizes, taking into consideration the changes in digestive physiology as the shrimp grow.

METHODOLOGIES COMMONLY USED FOR THE MEASUREMENT OF DIGESTIBILITY IN SHRIMP

Due to distinctive feeding habits and digestive physiology, determination of nutrient digestibility is more demanding with crustaceans than with fish species. But similar to what happens with finfish species, the first task in the measurement of digestibility of feeds and feedstuffs in shrimp is the collection of fecal samples. Nutrient digestibility can be measured gravimetrically by measuring feed intake and the subsequent fecal production, or indirectly by measuring the concentration of an inert marker in the feed and in the feces. However, measuring precisely the mass of feed ingested and of the feces produced, is difficult, particularly with shrimp which are slow-feeding animals. As a result digestibility measurements using direct methods involving total collection of fecal material are rarely used in aquatic species. The inert marker technique has become the method of choice in studies measuring nutrient digestibility in shrimp relying on the collection of a representative fecal sample (free of uneaten feed particles) and the use of a digestion indicator to obviate the need to quantify dietary intake and fecal output (indirect method). The inclusion of a digestion indicator in the diet allows the digestibility coefficients of the nutrients in a diet to be calculated from measurements of the nutrient-to-indicator ratios in the diet and feces. Chromic oxide and ytterbium acetate were found to be suitable digestibility markers for use in shrimp and provided as good or better results than the gravimetric technique (Smith and Tabrett, 2004). Yttrium oxide is also recognized as a suitable digestibility marker for use in fish as in shrimp.

Avoiding leaching losses from feed pellets prior to consumption and from feces prior to feces collection are among the big challenges faced when determining nutrient digestibility in aquatic species. Ingredient digestibility in shrimp has been determined passively by the use of:

I an indicator (innert marker) with Guelph or its modified systems and ingredients fed in the reference diet – IGIR;

II an indicator method (innert marker) with feces collected by siphoning and ingredients fed in the reference diet – ISPIR.

Techniques such as the periodical collection of feces by siphoning from the bottom of a tank are likely to yield inaccurate estimates of digestibility since the breakup of feces by fish or shrimp movement may lead to leaching of nutrients and, therefore, overestimation of digestibility of nutrients. Yet, collection of feces by siphoning from the bottom of the tank (Figure 2) has been the protocol most applied in studies with shrimp. Specific devices, developed by Ogino *et al.* (1973), Cho and Slinger (1979) and Choubert *et al.* (1979), to collect fecal material passively can help preventing these problems. Ogino *et al.* (1973) collected feces by passing the effluent water from fish tanks through a filtration column (TUF column). Cho and Slinger (1979) developed a settling column to separate the feces from the effluent water (Guelph system; Figure 3) and Choubert *et al.* (1979) developed a mechanically rotating screen to filter out fecal material (St. Pée system). During the last decades, these systems have been adopted in many laboratories around the world and widely recognized as producing meaningful estimates of digestibility of nutrients if used correctly. Although differences exist in the estimates of digestibility with the various techniques currently used, they tend to be fairly stable when these techniques are used in a standardized fashion.

Feed formulation for the determination of ingredient digestibility in shrimp, as seen in fish, has been following the protocol proposed by Cho and Slinger (1979) and later by Cho *et al.* (1982), comparing the digestibility of a reference diet with that of a test diet. In this protocol 7 parts (as is) of reference diet mash are mixed with 3 parts (as is) test ingredient to form a test diet. Major advantages of adopting this protocol are:

I formulation of nutritionally adequate test diets that can be produced with most potential aquafeed ingredients, and that allows the animals to maintain a high feed intake and good growth rate, which in turn allows the measurement of apparent digestibility values that are reliable and repeatable;

II measurement of feed intake and growth rate, allowing confirmation of the nutritional adequacy of the experimental diets.

Equation (Eq.) (1), proposed in the protocol of Cho and Slinger (1979) and later by Cho *et al.* (1982), is one of the most widely used for determining the digestibility of test ingredients for aquatic species.

Eq. (1) Apparent digestibility coefficient (ADC) of the test ingredients $= [\text{ADC}_{\text{test diet}} - (0.7 \text{ x } \text{ADC}_{\text{reference}} \text{ diet})] / 0.3$

However, and as discussed by Forster (1996) and Sugiura *et al.* (1996), this equation was shown to be mathematically incorrect. The later authors have demonstrated that Eq. (1) was mathematically incorrect since it did not account for the real nutrient contribution of the reference diet and the test ingredient. A revised equation (2) to calculate ADC of the test ingredient was first presented by Forster (1996) and published in peer-reviewed publications a few years later (Sugiura *et al.*, 1998; Forster, 1999):

Eq. (2) $\text{ADC}_{\text{ingredient}} = [(\text{ADC}_{\text{test diet}} \text{ x } D_{\text{test}}) - (0.7 \text{ x } D_{\text{ref}} \text{ x } \text{ADC}_{\text{reference diet}})] / (0.3 \text{ x } D_{\text{ing}})$

where

D_{ref} = % nutrient (or kJ/g gross energy) of reference diet (as is);

D_{test} = % nutrient (or kJ/g gross energy) of test diet (as is);

D_{ing} = % nutrient (or kJ/g gross energy) of test ingredient (as is).

Significant limitations to Eq. (2) have, however, been identified by Bureau *et al.* (1999) and discussed more recently by Bureau and Hua (2006). While Eq. (2) is mathematically correct, it assumes that $(0.7 \text{ x } D_{\text{ref}}) + (0.3 \text{ x } D_{\text{ing}}) = D_{\text{test}}$. This can only be accurate if the reference diet (pelleted), reference diet mash (unpelleted reference diet ingredient mixture combined with test ingredient in a 70:30 ratio), test ingredient and test diet (pelleted) all have the same dry matter (DM) content. Because this is almost never the case, a correction is needed to bring back all the terms on a comparable basis (i.e. comparable DM basis). If significant differences in DM content of the various components are present, the lack of such a correction will result in very significant bias in the estimate of the ADC of the test ingredient.

Therefore, the terms should be compared on the same basis as follows:

Eq. (3) $\text{ADC}_{\text{ingredient}} = [\text{ADC}_{\text{test diet}} \text{ x } D_{\text{test}} \text{ x } (0.7 \text{ x } \text{DM}_{\text{ref}} + 0.3 \text{ x } \text{DM}_{\text{ingr}}) - (0.7 \text{ x } D_{\text{ref}} \text{ x } \text{ADC}_{\text{reference diet}})] / (0.3 \text{ x } D_{\text{ingr}})$

where

DM_{ref} = % DM content of the reference diet "mash";

DM_{ingr} = % DM content of the test ingredient;

D_{test} = % nutrient (or kJ/g gross energy) of test diet (DM basis);

D_{ref} = % nutrient (or kJ/g gross energy) of reference diet "mash" (as is);

D_{ingr} = % nutrient (or kJ/g gross energy) of test ingredient (as is).

For the accurate use of Eq. 3, a careful sampling and chemical analysis of the reference diet mash becomes an essential component of the digestibility trials. This "mathematically correct" equation proposed by Bureau and Hua (2006) is now widely used for the determination of digestibility coefficients in fish and shrimp. One must, however, ensure that the nutrient level measured for a test diet are the same as what is predicted from the mash and test ingredient DM and nutrient levels (i.e. that $(0.7 \text{ x } D_{\text{ref}}) + (0.3 \text{ x } D_{\text{ing}}) = D_{\text{test}}$). If this is not the case, a very significant bias will be introduced in the ADC of test ingredient (the term solved for) as a result of amplification of error. Small errors are very common due to analytical, mixing or sampling error. If this occurs, samples should be reanalyzed or the use of a difference equation is necessary. This equation must partition the nutrient level in the test diet according to the theoretical nutrient contribution of the reference diet mash and test ingredient. This can be done as follows:

Eq. (4) $\mathrm{ADC}_{\text{test ingredient}} = \mathrm{ADC}_{\text{test diet}} + [(\mathrm{ADC}_{\text{test diet}} - \mathrm{ADC}_{\text{refdiet}}) \times (0.7 \times D_{\text{ref}} / 0.3 \times D_{\text{ingr}})]$

where

D_{ref} = % nutrient (or kJ/g gross energy) of reference diet mash (as is);

D_{ingr} = % nutrient (or kJ/g gross energy) of test ingredient (as is).

Variance of other components of the protocols being used to determine digestibility in shrimp has been analyzed by Smith and Tabrett (2004). The later authors have evaluated factors like:

I the effect of feed pellet processing on homogeneity of marker distribution;
II changes in feed pellet composition;
III comparison of different innert markers and the absorption of ingested chromic oxide;
IV leaching losses from feces;
V relative passage rates of nutrients and markers;
VI effect of feeding frequency on digestibility.

Detailed information about the effect of the above mentioned factors in the determination of digestibility in shrimp can be found in the study of Smith and Trabett (2004). Overall, the later authors have demonstrated that the level of homogeneity of the feed is a crucial factor to obtain reliable estimates of digestibility. A high level of homogeneity of the feed can minimize the number of replicate samples of feed that will need to be analyzed, the number of fecal samples that need to be collected, and the mass of feces (and hence number of days of collection) required for an accurate and precise estimate of feed digestibility. Reliable estimates of digestibility can be obtained if most of the feces from each treatment are collected over at least 5 days from eight replicate tanks, or for 10 days from six replicate tanks. Although the effect of shrimp development stage on digestibility has not been fully explored, according to Smith *et al.* (1985) the digestibility of feeds in small (8.4 to 11 g), medium (12.8 to 16.3 g) and large (22.8 to 25.2 g) shrimp is similar over this size range (8 to 25 g) The number of days that feces are collected would have, however, to be increased when smaller shrimp (<10 g) are used, due to the reduced mass of fecal output. Smith and Trabett (2004) have shown that both, chromic oxide and ytterbium, can be used as valid inert markers in digestibility experiments. Finally, comparisons between the gravimetric method and various markers in the study of Smith and Trabett (2004) showed that the results obtained with protocols using inert markers to measure digestibility in shrimp are less susceptible to error.

The percentage of nutrients lost by leaching in seawater before diet ingestion is not the focus of this review but can be estimated as described by Cruz-Suárez *et al.* (2007).

SHORT-REVIEW OF AVAILABLE NUTRIENT DIGESTIBILITY VALUES IN WHITELEG SHRIMP

Although the amount of nutrient digestible data available for shrimp is still limited, the number of studies reporting nutrient digestibility, including amino acid digestibility, has increased significantly during the last decade and are summarized in Table 1. The proximate and, whenever available, the respective amino acid composition of each of the different ingredients evaluated for their nutrient digestibility (Table 1) is provided as Annex 1. Detailed information about the experimental condition, under which the different studies reviewed here were conducted, is provided in Annex 2.

As can be seen in Table 1, digestibility coefficients of DM and CP are by far the most commonly reported in shrimp. Information about amino acid digestibility in white leg shrimp is now available for several ingredients, including those commonly used as alternative protein sources to fish meal.

Figure 2 Digestibility system at CSIRO, Australia (image: Courtesy of Dr. Stuart Arnold, CSIRO, Australia)

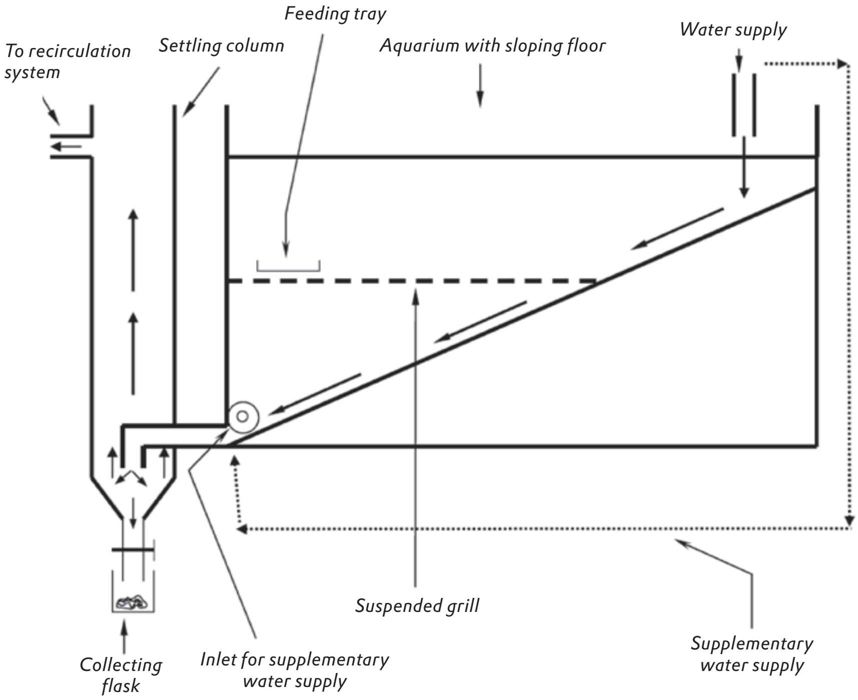


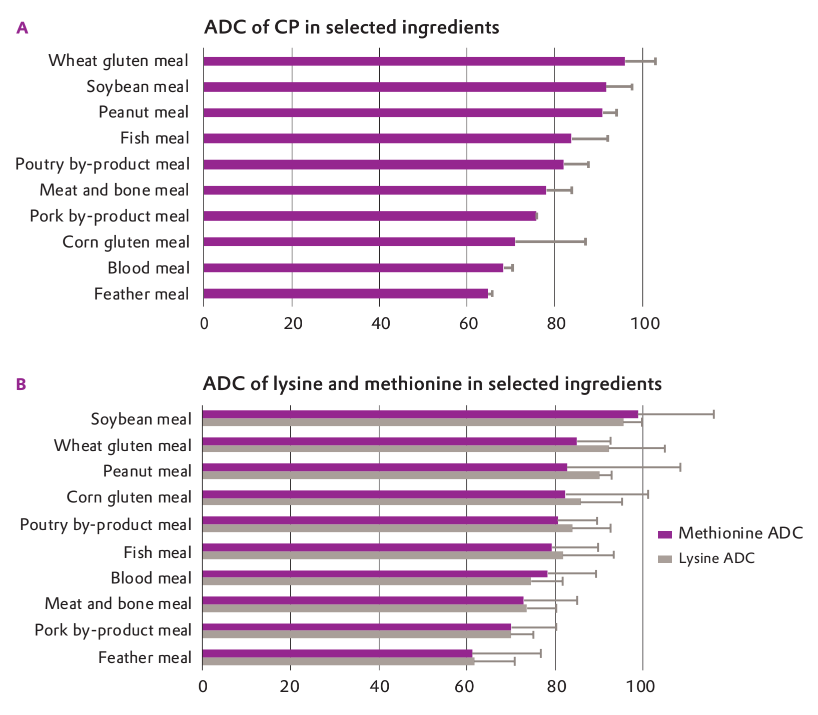
Figure 3 Guelph system modified for the collection of shrimp feces (source: Hernández *et al.* (2008))

Digestibility coefficients for individual essential amino acids, except tryptophan, have been reported for ingredients like blood meal (Villarreal-Cavazos *et al.*, 2014; Liu *et al.*, 2013), corn gluten meal (Yang *et al.*, 2009; Liu *et al.*, 2013), cottonseed meal (Liu *et al.*, 2013), extruded soybean meal (Yang *et al.*, 2009), feather meal (Villarreal-Cavazos *et al.*, 2014), fermented soybean meal (Yang *et al.*, 2009), fish meal of different origins and sources (Yang *et al.*, 2009; Terrazas-Fierro *et al.*, 2010; Liu *et al.*, 2013), full fat soybean meal (Cruz-Suarez *et al.*, 2009), meat and bone meal (Yang *et al.*, 2009; Liu *et al.*, 2013), peanut meal (Yang *et al.*, 2009; Liu *et al.*, 2013), plasma protein meal (Yang *et al.*, 2009), pork by-product meal (Terrazas *et al.*, 2010; Villarreal-Cavazos *et al.*, 2014), poultry by-product meal (Yang *et al.*, 2009; Terrazas *et al.*, 2010; Liu *et al.*, 2013, Villarreal-Cavazos *et al.*, 2014), rapeseed meal (Liu *et al.*, 2013), different shrimp by-product meals (Yang *et al.*, 2009; Terrazas-Fierro *et al.*, 2010; Liu *et al.*, 2003), soybean meal (Cruz-Suarez *et al.*, 2009; Yang *et al.*, 2009; Terrazas *et al.*, 2010; Liu *et al.*, 2013), soy protein concentrate (Cruz-Suarez *et al.*, 2009), different squid meals (Terrazas-Fierro *et al.*, 2010; Liu *et al.*, 2013) and wheat gluten meal (Yang *et al.*, 2009; Terrazas *et al.*, 2010). For many of these ingredients, also the digestibility of the non-essential amino acids is given (Table 1).

Figure 4 shows the average ± standard deviation for digestibility coefficients of CP (Figure 4 A), lysine and methionine (Figure 4 B) for 10 of all of the ingredients included in Table 1, and for which at least two measurements are given. The large variability found in literature for ADC of CP, but especially of amino acids like lysine and methionine, reported within the same ingredient, is well illustrated in Figure 4. Although the factors behind this large variability are yet to be fully understood, it can be at least partly attributed to the use of different methodological approaches for determination of ADC in shrimp. Standardization of such methodological approaches will positively contribute to the determination of more accurate ADC and thus, for the accuracy in feed formulation. Of the 10 ingredients we could evaluate, feather meal was the one showing the poorest CP, lysine and methionine digestibility values. In average, the highest CP, lysine and methionine digestibility coefficients are found for wheat gluten meal, soybean and peanut meal, while for all the other ingredients there seems to be no consistent relationship between CP and lysine or methionine digestibility coefficients.

The digestible content of CP, lysine and methionine in these same ingredients relatively to fish meal (taken as reference; 100 %) is shown in Figure 5. Compared to fish meal, available CP content is low in all of the 10 ingredient analyzed with the exception of blood meal, feather meal and wheat gluten. Lysine and methionine contents are both limiting in feather meal, meat and bone meal, peanut meal, pork by-product meal, being in the case of feather meal and peanut meal severely limiting (< 45 %). Results found for feather meal illustrate well the existence of discrepancies between available CP and essential amino acid contents, and the need of moving away from using total or even digestible CP content as a single criterion in the evaluation of ingredient quality. The digestible content of lysine is also limiting in corn gluten meal (< 40 %) and wheat gluten meal (< 45 %), and digestible content of methionine limiting in soybean meal (< 65 %), blood meal (< 80 %) and slightly limiting in poultry by-product meal (90 %). On the other hand, ingredients like corn gluten meal and wheat gluten meal have a relatively high content of digestible methionine and blood meal of digestible lysine.

Figure 4 Average ± standard deviation for ADC of CP (Figure 4 A), lysine and methionine (Figure 4 B) for 10 of all of the ingredients included in Table 1, and for which at least two measurements are available.



Despite the limited number of studies reporting amino acid digestibility coefficients for a given ingredient and thus not allowing meta-analyses to be performed, regression of total against nutrient digestible values was performed in an attempt to evaluate the use of crude total nutrient values to estimate digestible nutrient values of selected ingredients. This analysis has been performed with the total vs. digestible values of CP, lysine and methionine, available for fish meal and poultry by-product meal. Here, CP, lysine and methionine were selected for being of the most important nutrients in defining feedstuff quality.

Figure 5 Relative digestible content of CP, lysine and methionine relatively to fish meal (100 %) of 10 of all of the ingredients included in Table 1, and for which at least two measurements are available. The digestible content is calculated by multiplying nutrient content (ANNEX 1) by respective nutrient digestibility coefficient (Table 1).

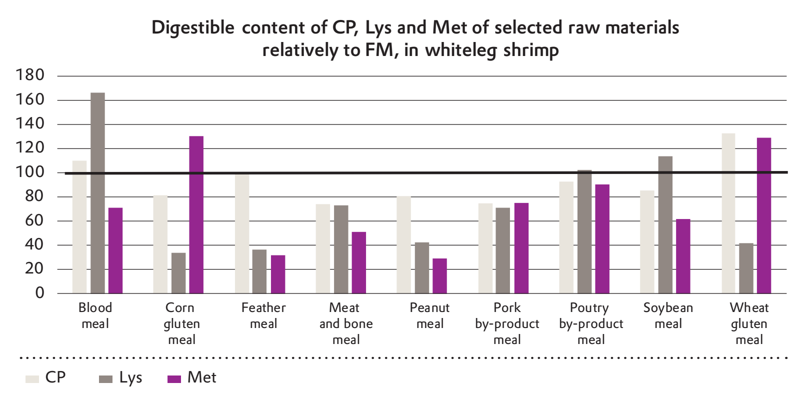
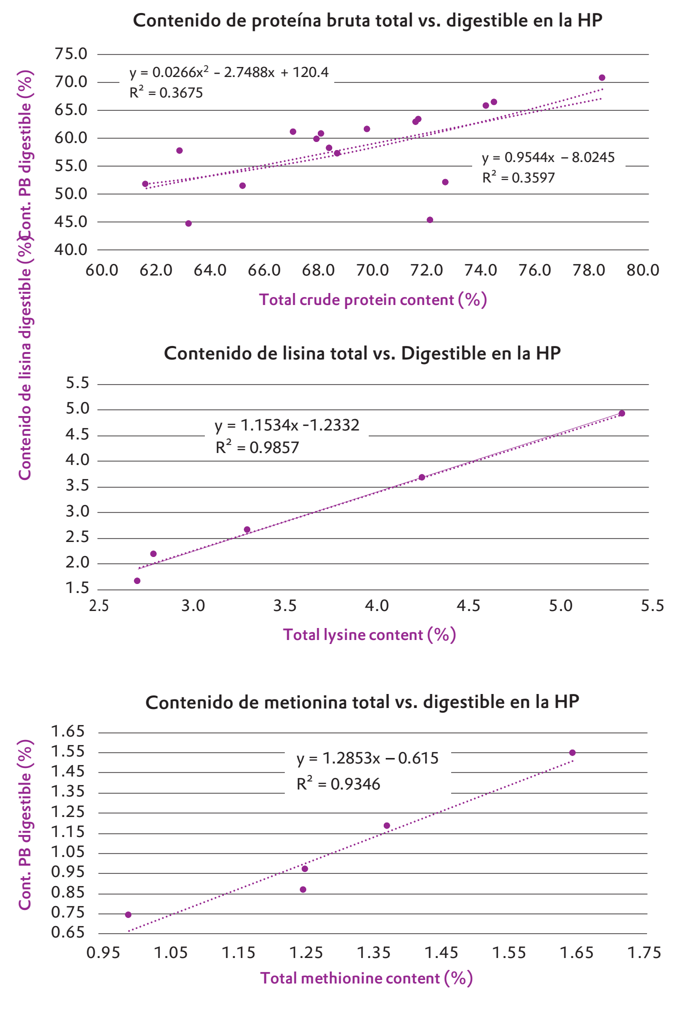


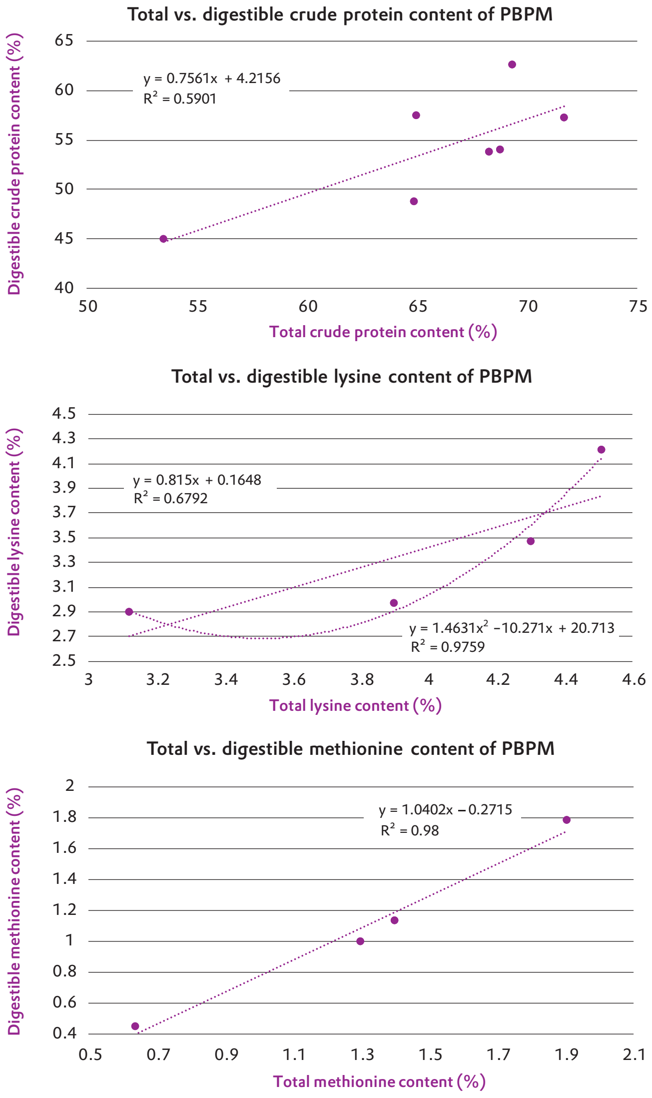
Figure 6 Regression of total against digestible CP, lysine and methionine contents determined for fish meal. Digestible content is calculated by multiplying nutrient content (ANNEX 1) by respective nutrient digestibility coefficient (Table 1). Respective equations and coefficients of determination are given in the figure.



Fish meal and poultry by-product meal were selected for being among all ingredients evaluated those with a higher number of measurements. Regression analyses done for fish meal (Figures 6 and 8) and poultry by-product meal values (Figures 7 and 9) illustrate the relationship between:

- total and digestible CP, total and digestible lysine and total and digestible methionine values (Figures 6 and 7) and

Figure 7 Regression of total against digestible CP, lysine and methionine contents determined for poultry by-product meal. Digestible content is calculated by multiplying nutrient content (ANNEX 1) by respective nutrient digestibility coefficient (Table 1). Respective equations and coefficients of determination are given in the figure.



- digestible CP and digestible lysine and digestible CP and digestible methionine values (Figures 8 and 9).

Seventeen measurements could be used for CP, 5 for lysine and methionine with fish meal. Seven measurements could be used for CP, 4 for lysine and methionine with poultry by-product meal. Regression analysis shows that, while total lysine and methionine content of fish meal fits relatively well with its respective digestible content (R^2 of 0.99 and 0.93 for lysine and methionine, respectively, obtained with linear regression models), the same is not true for CP when fitted to linear (R^2 of 0.36) or polynomial regression models (R^2 of 0.37) (Figure 6).

Figure 8 Regression of digestible CP against digestible lysine and methionine contents determined for fish meal. Digestible content is calculated by multiplying nutrient content (ANNEX 1) by respective nutrient digestibility coefficient (Table 1). Respective equations and coefficients of determination are given in the figure.

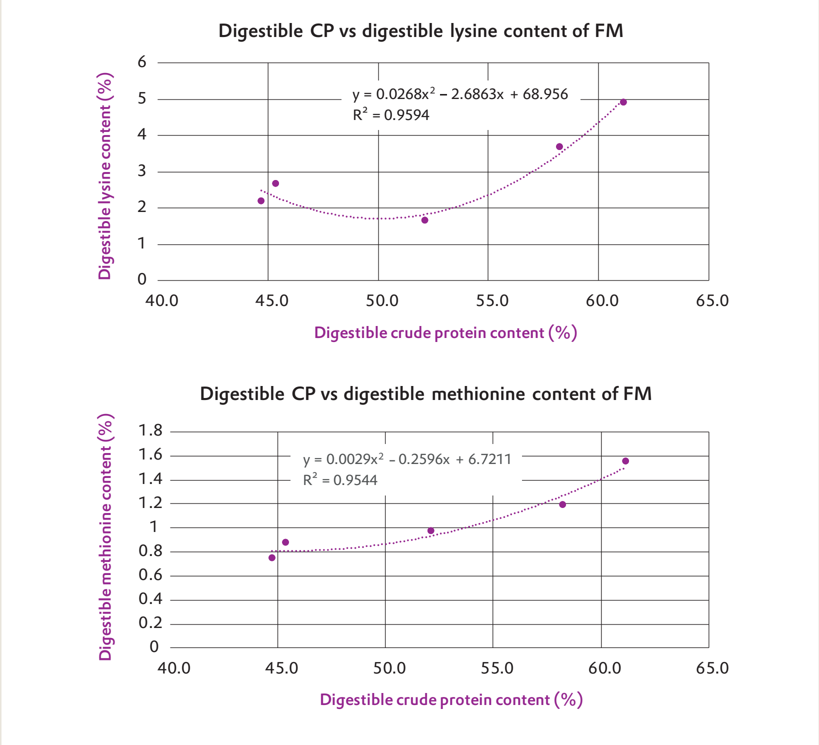
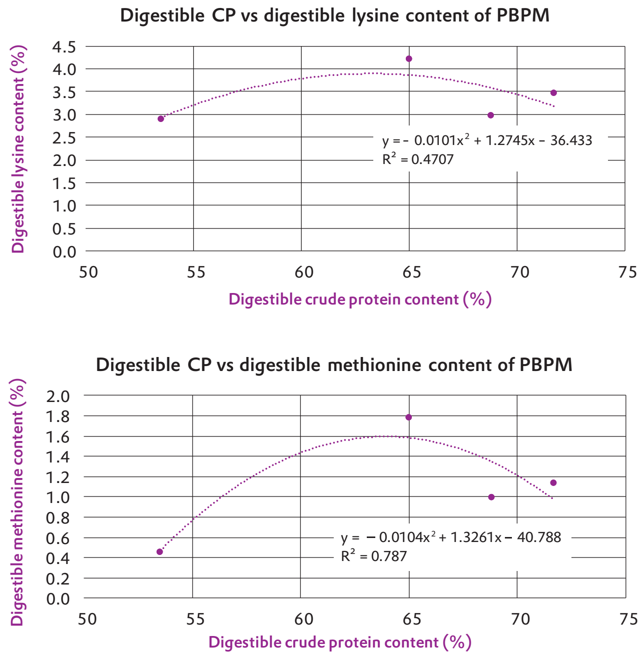


Figure 9 Regression of digestible CP against digestible lysine and methionine contents determined for poultry by-product meal. Digestible content is calculated by multiplying nutrient content (ANNEX 1) by respective nutrient digestibility coefficient (Table 1). Respective equations and coefficients of determination are given in the figure.

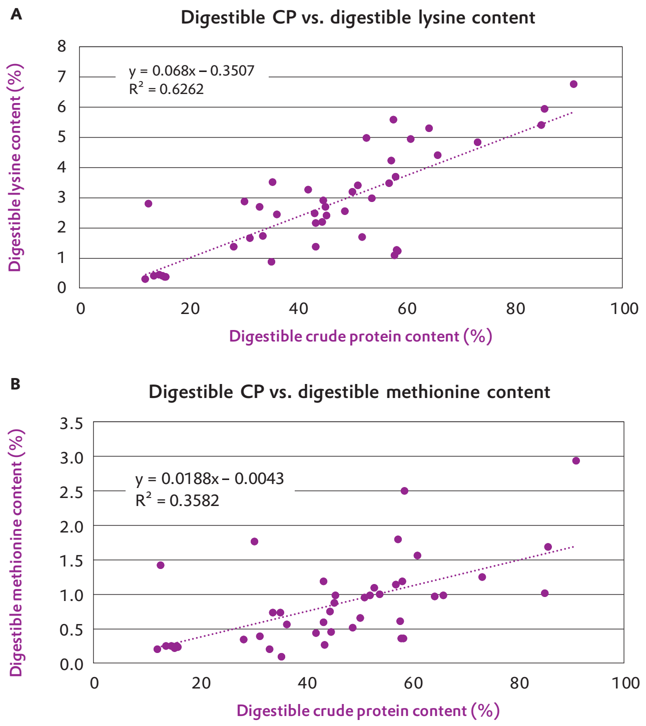


Similar results were found for poultry by-product meal. The relationship between total and digestible lysine and total and digestible methionine contents of poultry by-product meal is well described, respectively, by a polynomial (R^2 of 0.98) and linear (R^2 of 0.89) regression model (Figure 7).

Regression of digestible CP against digestible lysine and of digestible CP against digestible methionine values, determined for fish meal and poultry by-product meal, are shown in Figures 8 and 9, respectively.

The relationship between digestible CP and lysine and digestible CP and methionine were in both cases of polynomial nature. The limited number of digestibility measurements available for the two ingredients used in our regression analysis, fish meal and poultry by-product meal, obliges us to interpret the results with particular caution. Yet, results indicate digestible CP to be a good criterion to estimate lysine and methionine digestible contents of fish meal (R^2 of 0.96 and of 0.95 for lysine and methionine, respectively) but not of poultry by-product meal (R^2 of 0.47 and of 0.79 for lysine and methionine, respectively).

Figure 10 Regression of digestible CP against digestible lysine (Figure 10 A) or methionine (Figure 10 B) content, of all of the 46 digestibility measurements for which these values are both available. Digestible content is calculated by multiplying nutrient content (ANNEX 1) by respective nutrient digestibility coefficient (Table 1). Respective equations and coefficients of determination are given in the figure.



That the fit between digestible CP and digestible amino acid content is not consistent among ingredients, is also reflected in the poor relationship seen when digestible CP is regressed against digestible lysine (Figure 10 A; R^2 of 0.63) or methionine (Figure 10 B; R^2 of 0.36) content of all of the 46 measurements for which these values are both available. This might be at least partly attributed to the fact that CP level is calculated by multiplying the amount of nitrogen by the empirically derived conversion factor of 6.25, which is based on the estimation that protein contains 16 % nitrogen, although in reality it varies from 12 to 19 %. Thus, though digestibility of CP may be used to differentiate protein quality among ingredients, it does not allow shrimp nutritionists to accurately distinguish between nitrogen originating from amino acids and nitrogen originating from non-protein sources. Being a better criterion than total CP content, digestible protein content of an ingredient alone does not seem to be enough to accurately evaluate the quality of different ingredients. The use of accurate information on essential amino acid composition and digestibility is therefore a must for the development of cost-effective shrimp feeds. Further studies investigating the factors affecting nutrient, in particular amino acid digestibility of different ingredients in shrimp, would much contribute to the development of mathematical models estimating the digestible amino acid content in a more accurate way.

ACRONYMS

ADC	Apparent digestibility coefficient
CP	Crude protein
B-cells	Blister cells
DM	Dry matter
E-cells	Embryonic cells
Eq.	Equation
F-cells	Fibrillar cells
R-cells	Resorptive cells
PL	Postlarvae

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TABLE 1 Review of available information for DM, CP, crude lipid (CL), gross energy (GE) and amino acid ADC (%) for whiteleg shrimp

NO.	RAW MATERIAL	DM	CP	CL	GE	ARG	CYS	HIS	ILE	LEU	LYS	MET	PHE	THR	TYR	VAL	ALA	ASP	GLU	GLY	PRO	SER
1	**Blood meal**	57.50	67.10			69.30	58.80	65.70	68.50	64.10	69.50	74.60	67.00	67.00		65.90	65.70	67.00	68.00	67.60	63.90	65.50
2	**Blood meal**	55.20	69.10	70.20	57.50	80.90	77.30	58.40	87.60	77.70	79.60	90.10	87.40	73.40	65.70	68.50	68.20	79.60	75.80	98.80	80.80	82.40
3	**Blood meal**	57.00	66.20		72.20																	
4	**Blood meal (spray-dried)**	63.40	70.80		75.10																	
5	**Brewer's yeast**	71.70	85.70	72.10	84.60	91.30	89.90	90.50	92.20	90.50	91.00	30.30	48.20	80.10	95.40	84.40	81.30	84.10	95.20	62.40	98.40	87.70
6	**Casein**	97.30	99.90			101.60	100.10	102.40	100.60	97.80	99.70	100.00	100.30	99.70		100.40						
7	**Casein**	89.50	96.40		100.90																	
8	**Catarina scallop by-product meal**	67.20	86.80			94.70	87.20	97.50	83.20	86.00	89.00	89.30	84.90	82.50		85.10						
9	**Corn gluten meal**	82.80	81.20			96.70	87.60	92.90	84.90	75.90	75.30	88.30	70.70	83.40		81.50						
10	**Corn gluten meal**	41.80	59.10		65.40																	
11	**Corn gluten meal**	77.10	87.89	91.91	86.81	87.68	33.89	81.54	72.66	64.54	88.48	71.17	75.62	69.84	55.05	71.15	70.00	76.42	64.44	74.53	69.40	64.47
12	**Corn gluten meal**	48.60	55.70	67.30	51.10	86.20	88.40	69.90	62.40	52.40	93.70	50.60	23.80	71.50	15.60	64.10	50.20	78.40	44.50	97.80	42.50	65.60
13	**Cottonseed meal**	49.90	57.60	53.60	63.80	91.40	58.80	74.80	80.60	64.70	61.90	52.90	53.60	62.60	62.70	66.00	62.40	69.60	84.90	56.60	88.20	74.90
14	**Crab meal**	43.30	84.00		80.60																	
15	**Distillers grains**	47.20	78.50		69.60																	
16	**Durum wheat**	86.00	97.00		84.00	100.00	80.00	98.00	99.00	96.00	107.00	108.00	103.00	89.00		96.00	103.00	99.00	99.00	108.00	101.00	92.00
17	**Extruded soybean meal**	71.20	90.80	91.90	82.00	96.79	80.68	95.29	94.14	92.70	97.54	96.60	94.38	86.83	81.61	92.82	88.01	92.39	94.82	90.47	89.34	90.54
18	**Feather meal**	61.30	63.90		72.70																	
19	**Feather meal**	61.40	65.00			58.30	51.00	79.60	54.20	53.00	68.10	72.10	60.20	55.30		54.60	60.60	59.50	58.80	61.70	52.00	54.30
20	**Feather meal**	61.70	65.60			66.10	58.30	68.40	63.00	60.90	55.10	50.30	68.50	61.30		64.10	64.40	60.70	60.50	68.40	63.60	67.70
21	**Fermented soybean meal**	69.98	90.89	93.19	74.12	89.27	70.40	83.60	82.32	80.03	86.26	86.88	81.54	65.35	73.89	77.50	68.96	81.39	85.68	76.83	79.20	55.56
22	**Fish meal**	82.78	91.62	92.79	86.79	90.79	71.80	88.46	86.31	81.06	92.73	94.71	81.48	83.74	75.98	85.01	85.00	79.64	86.75	82.20	82.72	77.41
23	**Fish meal**	81.40	87.90		90.40																	
24	**Fish meal**	87.00	90.90	92.50	97.20	91.30	95.60	93.10	94.70	89.70	92.00	93.90	97.40	90.10	90.40	92.10	89.30	89.60	90.30	89.60	86.70	89.90
25	**Fish meal (sardine)**	76.20	84.90			98.80	89.20	96.60	92.10	92.40	86.20	86.20	79.40	86.40		86.90						
26	**Fish meal (sardine)**	52.20	71.50			81.90	68.70	81.20	75.20	72.10	61.40	77.30	64.90	78.90		73.90						
27	**Fish meal (sardine)**	44.00	62.70			78.20	69.70	83.50	69.60	72.10	80.40	69.40	64.70	66.80		69.20						
28	**Fish meal (tuna)**	52.60	70.50			79.30	61.50	84.00	70.40	73.50	78.10	74.90	65.10	65.20		69.50						
29	**Fish meal (anchovy)**	78.30	87.90		89.50																	
30	**Fish meal (anchovy)**	78.00	88.50		87.10																	
31	**Fish meal (herring)**	72.70	90.10		89.40																	
32	**Fish meal (hoki)**	67.10	88.10		88.80																	
33	**Fish meal (mackerel)**	73.50	88.80		88.30																	
34	**Fish meal (menhaden)**	68.10	89.00		88.40																	
35	**Fish meal (menhaden)**	55.60	83.70		83.30																	
36	**Fish meal (menhaden)**	60.20	83.20		86.70																	
37	**Fish meal**	55.80	78.60		81.30																	
38	**Fish meal**	70.70	87.60		87.30																	
39	**Fish soluble protein concentrate**	102.00	99.30			96.40	100.10	100.10	98.20	99.30	102.30	98.20	96.20	95.40		97.10						
40	**Full fat soybean meal**	82.70	95.70		88.10	99.30	94.10	96.90	95.90	94.90	98.20	97.90	96.10	93.50		95.30	98.30	97.20	97.60	101.80	99.20	94.60
41	**Gelatin**	96.50	99.70		102.20																	
42	**Hard red winter clear flour**	96.00	107.00		96.00	107.00	85.00	106.00	104.00	101.00	117.00	113.00	105.00	94.00		102.00	112.00	105.00	100.00	116.00	100.00	94.00
43	**Hard red winter wheat**	85.00	97.00		83.00	98.00	82.00	99.00	98.00	96.00	103.00	108.00	100.00	89.00		97.00	101.00	98.00	98.00	104.00	100.00	92.00
44	**Krill meal**	72.60	80.50		80.60																	
45	**Krill meal**	81.70	89.40		87.20																	
46	**Meat and bone meal**	56.33	73.88	83.72	84.80	68.59	64.39	71.58	71.70	64.17	68.77	81.48	65.93	64.41	45.68	65.46	70.17	64.66	70.22	81.89	68.58	58.90
47	**Meat and bone meal**	76.50	82.20	68.10	82.30	95.70	76.20	83.80	74.60	72.90	78.40	64.40	46.90	78.20	71.80	77.70	71.20	79.90	98.80	74.70	99.60	88.70
48	**Mixed wheat clear four**	91.00	99.00		96.00	91.00	82.00	94.00	90.00	91.00	94.00	94.00	98.00	84.00		89.00	88.00	86.00	97.00	87.00	96.00	89.00
49	**Peanut meal**	70.00	93.18	95.28	82.29	94.16	84.85	91.85	93.10	93.83	91.97	96.62	93.70	79.96	78.69	92.05	86.09	95.26	94.59	82.89	90.94	90.05
50	**Peanut meal**	53.20	88.80	77.80	72.00	99.90	99.80	86.40	99.20	87.80	88.10	60.10	74.10	91.50	88.30	85.90	82.60	95.30	96.30	85.00	94.40	90.10

Note: ADC > 100 % should be considered as 100 %

TABLE 1 Continuation

NO.	RAW MATERIAL	DM	CP	CL	GE	ARG	CYS	HIS	ILE	LEU	LYS	MET	PHE	THR	TYR	VAL	ALA	ASP	GLU	GLY	PRO	SER
51	**Plasma protein meal**	71.23	92.34	89.72	88.16	93.85	39.40	94.19	94.56	92.22	93.28	90.35	89.95	92.21	88.08	90.98	91.23	89.00	90.70	78.29	92.92	90.51
52	**Pork by-product meal**	68.20	75.80			82.60	72.50	80.00	81.50	78.20	66.20	87.80	66.80	79.40		74.00						
53	**Pork by-product meal**	68.80	75.70			72.90	36.20	76.40	64.10	64.40	73.60	73.40	68.40	64.40		66.40	75.40	70.70	71.00	79.20	76.30	60.00
54	**Poultry by-product meal**	72.00	83.90	66.80	84.00	95.40	71.70	91.00	93.50	85.40	92.90	70.80	54.90	88.40	87.90	91.50	89.10	86.50	94.70	92.10	95.20	91.60
55	**Poultry by-product meal**	90.80	90.40		93.30																	
56	**Poultry by-product meal**	89.00	88.30			94.00	85.00	80.80	87.40	89.00	93.50	93.60	86.60	84.90		89.70						
57	**Poultry by-product meal**	72.90	79.70			79.80	58.30	85.60	73.30	73.40	80.70	81.10	82.10	74.70		74.10	80.80	77.50	78.20	82.80	77.20	71.20
58	**Poultry by-product meal**	70.20	78.40			78.30	61.00	81.60	70.40	71.00	76.30	76.80	80.50	71.70		72.40	79.10	74.50	74.90	83.80	80.10	72.00
59	**Poultry by-product meal**	68.48	75.00	89.60	75.45	76.46	62.62	76.07	70.09	69.75	76.24	74.28	73.01	67.03	58.12	67.18	70.75	68.88	70.79	77.74	71.93	67.28
60	**Poultry by-product meal**	63.90	78.70		82.10																	
61	**Rapeseed meal**	50.80	78.30	54.30	65.60	93.10	82.20	85.80	87.10	82.20	82.90	64.60	62.60	80.50	84.30	81.60	83.80	79.00	89.80	85.30	99.60	86.60
62	**Rayon wheat**	84.00	99.00		83.00	101.00	82.00	101.00	97.00	95.00	106.00	110.00	104.00	90.00		95.00	105.00	101.00	99.00	116.00	103.00	95.00
63	**Red crab meal meal**	51.60	84.60			95.70	90.00	88.40	61.20	71.80	87.30	97.00	75.40	76.00		86.10						
64	**Semonila**	87.00	88.00		86.00	84.00	77.00	89.00	85.00	88.00	89.00	92.00	94.00	73.00		83.00	79.00	76.00	95.00	79.00	93.00	85.00
65	**Shrimp by-product meal**	52.83	84.71	91.61	72.32	92.52	78.75	89.40	86.48	87.53	90.51	89.58	87.02	78.70	74.48	87.93	81.03	86.07	86.33	81.58	86.53	82.48
66	**Shrimp head meal**	50.50	78.90	2.10	63.00	95.20	88.40	85.60	93.10	90.30	93.60	94.20	98.70	87.20	85.90	100.00	84.60	88.00	86.50	83.80	79.00	88.00
67	**Shrimp head meal**	84.00	98.00			98.30	93.70	97.80	93.30	97.40	105.40	95.60	89.80	93.70		94.70						
68	**Sorghum flour**	82.40	69.90			74.40	73.20	61.60	75.30	80.40	66.20	71.60	81.50	68.10		62.80						
69	**Soy protein isolate**	91.70	96.20		98.20	98.10	94.50	95.80	94.70	94.30	96.70	94.70	94.80	93.80		94.20	95.00	96.80	97.70	97.70	97.80	95.40
70	**Soybean meal**	71.70	82.30	75.20	83.00	99.10	85.20	97.90	98.90	85.80	99.40	60.70	98.30	91.30	88.70	78.40	87.10	95.60	93.10	88.90	85.80	93.40
71	**Soybean meal**	84.20	96.90		89.30	98.80	91.50	95.40	95.50	94.10	96.90	96.60	95.30	93.80		94.90	98.00	97.40	97.70	102.50	98.20	95.30
72	**Soybean meal**	85.40	100.00			103.60	94.90	101.00	99.50	100.30	96.30	97.90	87.20	92.80		99.30						
73	**Soybean meal**	75.90	92.90		85.60																	
74	**Soybean meal**	69.98	88.95	91.57	81.39	93.31	50.90	89.28	86.46	86.29	89.58	84.75	86.50	73.84	78.23	82.39	78.22	86.37	90.01	78.85	86.75	81.38
75	**Soybean meal**	78.70	93.70		95.00																	
76	**Soybean meal**	63.50	87.10		80.80																	
77	**Soybean protein concentrate**	82.60	93.00		85.10	95.30	86.40	91.00	89.50	88.10	92.90	92.40	88.90	86.70		88.70	92.40	92.50	93.50	96.70	93.70	90.00
78	**Squid meal (liver)**	61.80	66.40		74.00																	
79	**Squid meal**	68.60	84.50		67.60																	
80	**Squid meal**	61.90	75.40		78.50																	
81	**Squid meal (muscle)**	69.80	84.60		81.80																	
82	**Squid meal (muscle)**	74.70	86.60		84.10																	
83	**Squid meal**	95.00	95.40			99.50	92.70	97.10	85.80	94.20	94.40	90.40	85.30	87.10		90.70						
84	**Squid visceral meal**	51.60	70.90	85.50	66.80	90.10	84.10	83.20	90.10	69.30	93.00	58.30	64.70	81.70	71.20	74.30	85.00	85.50	85.70	86.20	81.00	83.90
85	**Wheat flour**	89.40	100.30			94.80	94.90	94.60	92.40	91.80	82.40	100.10	97.60	102.20		97.80						
86	**Wheat gluten meal**	109.20	103.10			108.40	101.70	103.40	103.10	103.60	101.20	104.10	98.90	98.90		99.80						
87	**Wheat gluten meal**	89.40	95.80		99.50																	
88	**Wheat gluten meal**	76.47	89.32	92.48	86.43	87.46	47.03	88.29	93.34	91.39	83.20	93.43	93.30	82.79	75.83	90.63	81.18	76.63	95.18	80.57	95.80	93.07
89	**Wheat starch**	92.30			98.90																	

Note: ADC > 100 % should be considered as 100 %

ANNEX 1 Dry matter, CP, crude lipid (CL), gross energy (GE) and amino acid composition (% diet or MJ/kg) of the different ingredients shown in Table 1

NO.	RAW MATERIAL	DM	CP	CL	GE	ARG	CYS	HIS	ILE	LEU	LYS	MET	PHE	THR	TYR	VAL	ALA	ASP	GLU	GLY	PRO	SER
1	Blood meal		95.90	2.20		5.00	1.70	4.90	3.80	9.10	7.60	1.30	5.70	4.70	6.20	6.90	8.40	9.80	3.60	4.00	4.40	65.50
2	Blood meal	93.10	83.67	0.75	20.95	3.16	0.60	4.35	0.99	8.37	7.00	0.68	5.35	3.34	5.67	6.80	10.04	6.49	4.37	2.02	3.77	82.40
3	Blood meal		97.60		24.02																	
4	Blood meal (spray-dried)		99.10		24.73																	
5	Brewer's yeast	91.60	41.48	4.37	19.98	2.35	0.36	1.09	1.04	2.70	3.86	0.28	1.53	2.51	1.80	2.41	5.14	5.53	2.19	1.00	2.22	87.70
6	Casein		91.20		15.74	3.38	0.59	2.91	5.21	9.06	6.78	2.93	5.10	3.95	6.30							
7	Casein		95.90		24.02																	
8	Catarina scallop by-product meal	95.50	52.57	15.18	19.26	3.88	0.57	0.44	1.76	2.61	2.69	1.09	1.25	1.04	1.63							
9	Corn gluten meal		72.30	2.50	22.33	2.68	1.49	1.83	3.82	13.60	1.63	2.82	5.64	2.74	3.97							
10	Corn gluten meal		71.60		23.72																	
11	Corn gluten meal	93.37	59.79	3.32	23.66																	64.47
12	Corn gluten meal	95.10	63.41	2.21	21.45	2.31	0.63	1.39	2.60	3.53	0.94	1.45	4.55	2.27	2.09	5.71	4.43	11.93	1.89	3.46	3.31	65.60
13	Cottonseed meal	93.30	49.20	1.61	18.54	4.94	0.57	1.63	1.30	2.44	2.23	0.63	2.29	1.88	1.73	2.15	5.96	7.61	2.71	1.29	2.43	74.90
14	Crab meal		33.30		11.05																	
15	Distillers grains		30.40		22.30																	
16	Durum wheat		14.20	1.90		0.66	0.30	0.34	0.49	0.97	0.38	0.23	0.66	0.38	0.63	0.48	0.69	4.03	0.52	1.37	0.63	92.00
17	Extruded soybean meal	87.72	45.79	2.82	20.46																	90.54
18	Feather meal		86.70		24.73																	
19	Feather meal		89.30	12.20		5.70	2.60	0.70	4.10	6.50	1.60	0.50	4.20	3.80	6.50	4.20	5.60	8.80	6.20	8.50	9.40	54.30
20	Feather meal		89.20	10.40		5.90	3.80	0.90	4.10	6.50	2.30	0.70	4.00	4.10	5.90	4.20	6.10	9.80	6.70	8.40	9.50	67.70
21	Fermented soybean meal	91.77	48.76	1.22	21.19																	55.56
22	Fish meal	93.11	63.07	8.86	21.89																	77.41
23	Fish meal	95.42	68.12	9.38	19.73	4.08	0.00	1.69	2.94	5.09	5.55	1.80	2.77	2.83	3.46							
24	Fish meal	92.60	67.28	6.26	20.09	4.17	0.57	1.60	2.47	4.74	5.36	1.65	1.98	3.37	2.46	4.46	7.85	7.95	6.13	2.04	3.41	89.90
25	Fish meal (sardine)	96.50	68.60	9.53	19.93	3.96	0.52	1.85	2.58	4.35	4.27	1.38	2.37	2.31	3.07							
26	Fish meal (sardine)	96.30	72.90	7.06	20.83	3.69	0.52	1.67	2.52	4.24	2.72	1.26	1.77	2.44	2.85							
27	Fish meal (sardine)	96.50	72.33	2.90	20.36	3.49	0.41	1.20	2.26	3.61	3.32	1.25	1.69	1.99	2.46							
28	Fish meal (tuna)	95.40	63.42	14.78	21.91	3.10	0.39	1.30	2.06	3.33	2.81	1.00	1.50	1.74	2.03							
29	Fish meal (anchovy)		70.00		21.59																	
30	Fish meal (anchovy)		74.40		19.96																	
31	Fish meal (herring)		78.70		22.18																	
32	Fish meal (hoki)		71.90		19.33																	
33	Fish meal (mackerel)		74.70		19.00																	
34	Fish meal (menhaden)		68.30		20.08																	
35	Fish meal (menhaden)		61.80		18.49																	
36	Fish meal (menhaden)		68.90		19.41																	
37	Fish meal		65.40		17.32																	
38	Fish meal		71.80		19.92																	
39	Fish soluble protein concentrate	92.00	86.30	3.48	21.81	7.99	0.84	1.57	3.98	6.18	5.80	1.72	3.16	3.35	3.64							
40	Full fat soybean meal	91.70	38.10	23.30	25.14	2.86	0.57	1.03	1.87	3.05	2.48	0.57	1.98	1.56	1.94	1.71	4.61	7.28	1.71	1.98	1.94	94.60
41	Gelatin		112.40		21.51																	
42	Hard red winter clear flour		14.70	1.10		0.55	0.32	0.31	0.50	0.97	0.31	0.22	0.71	0.38	0.59	0.44	0.58	4.79	0.51	1.63	0.67	94.00
43	Hard red winter wheat		15.10	2.70		0.68	0.33	0.35	0.49	0.98	0.41	0.23	0.68	0.43	0.60	0.52	0.75	4.31	0.61	1.46	0.69	92.00
44	Krill meal		70.20		21.71																	
45	Krill meal		62.80		22.89																	
46	Meat and bone meal	96.23	51.53	17.59	22.52																	58.90
47	Meat and bone meal	94.90	59.54	14.44	21.60	4.07	0.51	1.15	1.35	2.38	3.25	0.80	2.05	2.19	2.18	4.77	5.53	5.66	9.34	3.09	2.60	88.70
48	Mixed wheat clear four		16.00	2.60		0.71	0.33	0.37	0.52	1.03	0.39	0.24	0.73	0.43	0.66	0.52	0.71	4.76	0.61	1.59	0.71	89.00
49	Peanut meal	90.13	54.50	6.95	21.36																	90.05
50	Peanut meal	94.00	49.15	8.19	19.89	5.63	0.59	0.96	1.26	2.51	1.55	0.43	0.71	1.50	1.46	2.01	6.90	7.73	3.68	1.26	2.57	90.10

ANNEX 1

Continuation

NO.	RAW MATERIAL	DM	CP	CL	GE	ARG	CYS	HIS	ILE	LEU	LYS	MET	PHE	THR	TYR	VAL	ALA	ASP	GLU	GLY	PRO	SER
51	**Plasma protein meal**	93.14	66.86	1.80	19.68																	90.51
52	**Pork by-product meal**		57.30	9.50	17.93	7.16	0.79	1.71	3.23	5.78	3.73	1.34	3.26	2.90	4.26							
53	**Pork by-product meal**		57.40	12.40		3.90	0.50	1.10	1.60	3.10	2.90	0.80	1.80	1.80	2.30	4.00	4.20	6.90	6.40	4.60	2.20	60.00
54	**Poultry by-product meal**	95.50	53.51	18.64	20.63	3.84	0.27	1.18	0.94	2.52	3.12	0.64	2.12	2.19	2.35	3.41	5.40	5.96	5.43	1.88	2.13	91.60
55	**Poultry by-product meal**	95.59	69.36	13.18	22.32	4.61	0.00	1.55	2.55	4.69	4.15	1.30	2.58	2.59	3.15							
56	**Poultry by-product meal**		65.00	12.40	19.54	7.25	0.82	1.95	4.03	6.37	4.51	1.91	3.23	3.98	4.18							
57	**Poultry by-product meal**		71.70	15.40		4.80	0.70	1.60	2.70	4.50	4.30	1.40	2.60	2.70	3.20	4.60	5.70	9.00	6.10	4.30	2.80	71.20
58	**Poultry by-product meal**		68.80	16.10		4.40	0.70	1.60	2.50	4.30	3.90	1.30	2.50	2.60	3.00	4.30	5.30	8.40	5.70	4.20	3.00	72.00
59	**Poultry by-product meal**	91.07	64.90	12.60	20.97																	67.28
60	**Poultry by-product meal**		68.30		20.79																	
61	**Rapeseed meal**	91.20	40.13	3.29	19.08	2.79	0.52	1.30	1.17	2.25	1.97	0.59	1.36	2.08	1.59	2.07	3.59	6.47	2.68	1.66	2.03	86.60
62	**Rayon wheat**		15.50	2.00		0.67	0.33	0.35	0.49	0.97	0.39	0.20	0.71	0.42	0.60	0.51	0.75	4.58	0.60	1.52	0.71	95.00
63	**Red crab meal meal**	95.50	39.90	4.50	14.88	3.28	0.35	0.98	1.08	2.02	1.97	0.75	1.19	1.17	1.72							
64	**Semonila**		13.70	2.50		0.55	0.28	0.32	0.49	0.95	0.31	0.22	0.66	0.36	0.58	0.41	0.58	4.23	0.45	1.46	0.62	85.00
65	**Shrimp by-product meal**	88.89	49.90	1.88	20.97																	82.48
66	**Shrimp head meal**	96.20	38.57	1.77	12.68	2.84	0.28	0.84	0.97	1.76	3.07	1.87	0.52	1.82	1.86	2.36	4.76	4.68	2.78	1.06	1.77	88.00
67	**Shrimp head meal**	95.30	52.26	3.67	17.11	4.85	0.44	0.77	2.44	3.53	3.21	0.99	2.19	1.74	2.56							
68	**Sorghum flour**		8.40	3.90	16.88	5.12	1.67	2.02	4.05	13.69	7.26	1.67	5.60	2.98	5.60							
69	**Soy protein isolate**	92.20	88.50	0.10	22.40	6.73	1.06	2.30	4.25	7.17	5.58	1.06	4.78	3.45	4.25	3.81	10.80	17.79	3.81	4.69	4.78	95.40
70	**Soybean meal**	92.70	51.13	1.29	19.63	3.94	0.80	1.41	1.57	3.00	3.26	0.70	1.94	2.24	1.73	2.25	6.75	7.32	2.48	1.56	2.70	93.40
71	**Soybean meal**	91.10	52.00	2.00	19.14	3.85	0.73	1.35	2.34	4.06	3.28	0.68	2.65	2.13	2.44	2.29	6.08	9.72	2.24	2.60	2.70	95.30
72	**Soybean meal**		52.90	2.60	17.92	6.56	1.04	2.57	4.10	6.77	5.16	1.12	4.67	3.33	4.27							
73	**Soybean meal**		51.60		18.49																	
74	**Soybean meal**	90.29	39.98	17.88	23.13																	81.38
75	**Soybean meal**		89.60		22.51																	
76	**Soybean meal**		42.50		23.26																	
77	**Soybean protein concentrate**	91.50	70.90	0.70	20.35	5.60	0.99	1.99	3.40	5.81	4.75	1.06	3.76	2.98	3.55	3.26	8.72	13.83	3.19	3.76	93.70	90.00
78	**Squid meal (liver)**		53.50		22.30																	
79	**Squid meal**		88.90		23.47																	
80	**Squid meal**		73.00		19.79																	
81	**Squid meal (muscle)**		91.40		23.56																	
82	**Squid meal (muscle)**		90.10		23.81																	
83	**Squid meal**	92.50	76.97	3.57	20.34	5.88	0.63	1.64	2.90	5.56	5.11	1.38	2.46	2.64	2.99							
84	**Squid visceral meal**	86.80	46.77	18.32	23.16	2.62	0.47	1.18	1.12	3.31	2.87	0.35	1.00	1.99	1.79	3.43	4.55	7.97	2.82	1.96	81.00	83.90
85	**Wheat flour**		12.70	0.70	15.76	3.86	1.81	2.20	4.02	6.06	3.39	1.42	4.25	2.36	4.49							
86	**Wheat gluten meal**		83.20	1.50	21.07	3.10	1.94	1.94	3.80	6.35	1.48	1.50	5.19	2.32	3.81							
87	**Wheat gluten meal**		83.70		23.64																	
88	**Wheat gluten meal**	91.32	74.89	1.81	23.61																95.80	93.07
89	**Wheat starch**				17.45																	

ANNEX 2

Detailed information, whenever available, on the methodological approach used to determine ADCs of the different ingredients shown in Table 1

NO.	RAW MATERIAL	REFERENCE	PROTOCOL		PELLET Ø, MM	FEED TYPE	BW, G	TEMP, °C	SALINITY, PPT	REP.	N° MEALS	SAMPLING TIME*	TRIAL DURATION**
1	**Blood meal**	Villarreal-Cavazos *et al.*, 2014	ISPIR	a	1.6	Spaghetti-like strands	5.1			4			7
2	**Blood meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
3	**Blood meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.09	30.1	32.2	3	6	6 (1h)	4
4	**Blood meal (spray-dried)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.09	30.1	32.2	3	6	6 (1h)	4
5	**Brewer's yeast**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
6	**Casein**	Terrazas *et al.*, 2010	ISPIR	a	2	Spaghetti-like strands	16.5	27	39	3	3	2 (1h, 2h)	45
7	**Casein**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.09	30.1	32.2	3	6	6 (1h)	4
8	**Catarina scallop by-product meal**	Terrazas-Fierro *et al.*, 2010	ISPIR	a	2	Pelleted	15-19	27	40	3	4	3 (1h)	
9	**Corn gluten meal**	Terrazas *et al.*, 2010	ISPIR	a	2	Spaghetti-like strands	16.5	27	39	3	3	2 (1h, 2h)	45
10	**Corn gluten meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.09	30.1	32.2	3	6	6 (1h)	4
11	**Corn gluten meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28–30.5	30	3	4	3 (1.5h)	42
12	**Corn gluten meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
13	**Cottonseed meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
14	**Crab meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.09	30.1	32.2	3	6	6 (1h)	4
15	**Distillers grains**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.09	30.1	32.2	3	6	6 (1h)	4
16	**Durum wheat**	Nieto-López *et al.*, 2011	ISPIR	a	0.5	Spaghetti-like strands	3.56	29.8	26.2	4	3	6 (2h, 3h 15m)	15
17	**Extruded soybean meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28–30.5	30	3	4	3 (1.5 h)	42
18	**Feather meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.09	30.1	32.2	3	6	6 (1h)	4
19	**Feather meal**	Villarreal-Cavazos *et al.*, 2014	ISPIR	a	1.6	Spaghetti-like strands	5.1			4			7
20	**Feather meal**	Villarreal-Cavazos *et al.*, 2014	ISPIR	a	1.6	Spaghetti-like strands	5.1			4			7
21	**Fermented soybean meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28–30.5	30	3	4	3 (1.5 h)	42
22	**Fish meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28–30.5	30	3	4	3 (1.5 h)	42
23	**Fish meal**	Cruz-Suarez *et al.*, 2007	ISPIR	a		Spaghetti-like strands	2.6	27–31	24–30	4	2	2 (1.5h, 2h)	7
24	**Fish meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
25	**Fish meal (sardine)**	Terrazas-Fierro *et al.*, 2010	ISPIR	a	2	Pelleted	15-19	27	40	3	4	4 (1h)	
26	**Fish meal (sardine)**	Terrazas-Fierro *et al.*, 2010	ISPIR	a	2	Pelleted	15-19	27	40	3	4	4 (1h)	
27	**Fish meal (sardine)**	Terrazas-Fierro *et al.*, 2010	ISPIR	a	2	Pelleted	15-19	27	40	3	4	4 (1h)	
28	**Fish meal (tuna)**	Terrazas-Fierro *et al.*, 2010	ISPIR	a	2	Pelleted	15-19	27	40	3	4	4 (1h)	
29	**Fish meal (anchovy)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.09	30.1	32.2	3	6	6 (1h)	4
30	**Fish meal (anchovy)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.10	30.1	32.2	3	6	6 (1h)	4
31	**Fish meal (herring)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.11	30.1	32.2	3	6	6 (1h)	4
32	**Fish meal (hoki)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.12	30.1	32.2	3	6	6 (1h)	4
33	**Fish meal (mackerel)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.13	30.1	32.2	3	6	6 (1h)	4
34	**Fish meal (menhaden)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.14	30.1	32.2	3	6	6 (1h)	4
35	**Fish meal (menhaden)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.15	30.1	32.2	3	6	6 (1h)	4
36	**Fish meal (menhaden)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.16	30.1	32.2	3	6	6 (1h)	4
37	**Fish meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.17	30.1	32.2	3	6	6 (1h)	4
38	**Fish meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.17	30.1	32.2	3	6	6 (1h)	4
39	**Fish soluble protein concentrate**	Terrazas-Fierro *et al.*, 2010	ISPIR	a	2	Pelleted	15-19	27	40	3	4	4 (1h)	
40	**Full fat soybean meal**	Cruz-Suarez *et al.*, 2009	ISPIR	a		Spaghetti-like strands	5.9	29.8	26.2	4	3	6 (2h, 3h 15m)	15
41	**Gelatin**	Siccardi III *et al.*, 2006	ISPIR	a			11.33			3			
42	**Hard red winter clear flour**	Nieto-López *et al.*, 2011	ISPIR	a	0.5	Spaghetti-like strands	3.56	29.8	26.2	4	3	6 (2h, 3h 15m)	15
43	**Hard red winter wheat**	Nieto-López *et al.*, 2011	ISPIR	a	0.5	Spaghetti-like strands	3.56	29.8	26.2	4	3	6 (2h, 3h 15m)	15
44	**Krill meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.17	30.1	32.2	3	6	6 (1h)	4
45	**Krill meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65-15.17	30.1	32.2	3	6	6 (1h)	4

BW = body weight
Rep. = Number of replicates
* Sampling time is given as the n° of feces collections per day (postprandial collection time)
** Trial duration is given as the number of feces collection days
IGIR = Determined by indicator method (innert marker) with Guelph or its modified systems and ingredient fed in reference diet
ISPIR = Determined by indicator method (innert marker) with feces collected by siphoning, and ingredient fed in reference diet
a = Equation used as described by Bureau and Hua (2006)
b = Equation used as described by Cho *et al.* (1982)

ANNEX 2

Continuation

NO.	RAW MATERIAL	REFERENCE	PROTOCOL		PELLET Ø, MM	FEED TYPE	BW, G	TEMP, °C	SALINITY, PPT	REP.	N° MEALS	SAMPLING TIME*	TRIAL DURATION**
46	**Meat and bone meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28 – 30.5	30	3	4	3 (1.5h)	42
47	**Meat and bone meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
48	**Mixed wheat clear four**	Nieto - López *et al.*, 2011	ISPIR	a	0.5	Spaghetti - like strands	3.56	29.8	26.2	4	3	6 (2h, 3h 15m)	15
49	**Peanut meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28 – 30.5	30	3	4	3 (1.5h)	42
50	**Peanut meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
51	**Plasma protein meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28–30.5	30	3	4	3 (1.5h)	42
52	**Pork by-product meal**	Terrazas *et al.*, 2010	ISPIR	a	2	Spaghetti-like strands	16.5	27	39	3	3	2 (1h, 2h)	45
53	**Pork by-product meal**	Villarreal-Cavazos *et al.*, 2014	ISPIR	a	1.6	Spaghetti-like strands	5.1			4			7
54	**Poultry by-product meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
55	**Poultry by-product meal**	Cruz-Suarez *et al.*, 2007	ISPIR	a		Spaghetti-like strands	2.6	27–31	24-30	4	2	2 (1.5h, 2h)	7
56	**Poultry by-product meal**	Terrazas *et al.*, 2010	ISPIR	a	2	Spaghetti-like strands	16.5	27	39	3	3	2 (1h, 2h)	45
57	**Poultry by-product meal**	Villarreal-Cavazos *et al.*, 2014	ISPIR	a	1.6	Spaghetti-like strands	5.1			4			7
58	**Poultry by-product meal**	Villarreal-Cavazos *et al.*, 2014	ISPIR	a	1.6	Spaghetti-like strands	5.1			4			7
59	**Poultry by-product meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28–30.5	30	3	4	3 (1.5 h)	42
60	**Poultry by-product meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4
61	**Rapeseed meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
62	**Rayon wheat**	Nieto-López *et al.*, 2011	ISPIR	a	0.5	Spaghetti-like strands	3.56	29.8	26.2	4	3	6 (2h, 3h 15m)	15
63	**Red crab meal meal**	Terrazas-Fierro *et al.*, 2010	ISPIR	a	2	Pelleted	15 – 19	27	40	3	4	4 (1h)	
64	**Semonila**	Nieto-López *et al.*, 2011	ISPIR	a	0.5	Spaghetti-like strands	3.56	29.8	26.2	4	3	6 (2h, 3h 15m)	15
65	**Shrimp by-product meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28–30.5	30	3	4	3 (1.5h)	42
66	**Shrimp head meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
67	**Shrimp head meal**	Terrazas-Fierro *et al.*, 2010	ISPIR	a	2	Pelleted	15 – 19	27	40	3	4	4 (1h)	
68	**Sorghum flour**	Terrazas *et al.*, 2010	ISPIR	a	2	Spaghetti-like strands	16.5	27	39	3	3	2 (1h, 2h)	45
69	**Soy protein isolate**	Cruz-Suarez *et al.*, 2009	ISPIR	a		Spaghetti-like strands	5.9	29.8	26.2	4	3	6 (2h, 3h 15m)	15
70	**Soybean meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
71	**Soybean meal**	Cruz-Suarez *et al.*, 2009	ISPIR	a		Spaghetti-like strands	5.9	29.8	26.2	4	3	6 (2h, 3h 15m)	15
72	**Soybean meal**	Terrazas *et al.*, 2010	ISPIR	a	2	Spaghetti-like strands	16.5	27	39	3	3	2 (1h, 2h)	45
73	**Soybean meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.2	30.1	32.2	3	6	6 (1h)	4
74	**Soybean meal**	Siccardi III *et al.*, 2006	IGIR	b	1.5	Wet extruded	1.05	28–30.5	30	3	4	3 (1.5h)	42
75	**Soybean meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4
76	**Soybean meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4
77	**Soybean protein concentrate**	Cruz-Suarez *et al.*, 2009	ISPIR	a		Spaghetti-like strands	5.9	29.8	26.2	4	3	6 (2h, 3h 15m)	15
78	**Squid meal (liver)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4
79	**Squid meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4
80	**Squid meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4
81	**Squid meal (muscle)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4
82	**Squid meal (muscle)**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4
83	**Squid meal**	Terrazas-Fierro *et al.*, 2010	ISPIR	a	2	Pelleted	15 – 19	27	40	3	4	4 (1h)	
84	**Squid visceral meal**	Liu *et al.*, 2013	ISPIR	b	1.5	Pelleted	4.45	26		3	2	2 (1h)	42
85	**Wheat flour**	Terrazas *et al.*, 2010	ISPIR	a	2	Spaghetti-like strands	16.5	27	39	3	3	2 (1h, 2h)	45
86	**Wheat gluten meal**	Terrazas *et al.*, 2010	ISPIR	a	2	Spaghetti-like strands	16.5	27	39	3	3	2 (1h, 2h)	45
87	**Wheat gluten meal**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4
88	**Wheat gluten meal**	Yang *et al.*, 2009	IGIR	b	1.5	Wet extruded	1.05	28–30.5	30	3	4	3 (1.5h)	42
89	**Wheat starch**	Siccardi III *et al.*, 2006	ISPIR	a	2	Spaghetti-like strands	8.65 – 15.17	30.1	32.2	3	6	6 (1h)	4

BW = body weight
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* Sampling time is given as the n° of feces collections per day (postprandial collection time)
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a = Equation used as described by Bureau and Hua (2006)
b = Equation used as described by Cho *et al.* (1982)

THE POTENTIAL FOR FEEDING LOW CRUDE PROTEIN-AMINO ACID SUPPLEMENTED DIETS TO STARTER AND GROWING-FINISHING PIGS

DR. JOHN HTOO

KEY INFORMATION

- Crude protein levels in pig diets can be reduced by at least 4 %-points without affecting performance, carcass quality or nitrogen retention by balancing for adequate levels of all essential amino acids on standardized ileal digestible basis and dietary energy preferably on net energy basis.
- When dietary crude protein is reduced by 5 %-points or more, a maximum dietary standardized ileal digestible lysine:crude protein ratio of 6.9 % (7.4 % total lysine:crude protein ratio) should be kept by supplementing with some nonessential amino acids such as glycine and glutamate in addition to balancing all essential amino acids.
- On average, every 1 %-point dietary crude protein reduction results in approximately 9 % reduction in nitrogen excretion in pigs.
- The availability of supplemental amino acids allows nutritionists to formulate low crude protein diets that are cost effective and more environmentally friendly.

INTRODUCTION

Feeding pigs with high protein cereals-soybean meal based diets is rather inefficient in term of protein or nitrogen (N) utilization because about 60 % of ingested N is excreted with urine and feces (Le Bellego *et al.*, 2001). As such, feeding diets with excess crude protein (CP) level is the main cause of N pollution. Research has consistently showed that lowering the dietary CP level and balancing with supplemental amino acids (AA) is a very effective strategy to reduce N excretion. With feed-grade L-Valine (Val) now commercially available, nutritionists have a greater flexibility to further reduce the dietary CP level and to meet animal requirements more precisely.

High feed cost has been a major challenge to profitability for pork producers in recent years. Considering that feed cost is the biggest portion, as much as 70 %, of the overall variable pig production cost, then replacing a portion of the AA in the protein sources such as soybean meal with supplemental AA improves the efficiency of nutrient utilization and more importantly it is cost effective.

Due to the increased public awareness and governmental regulations to reduce environmental pollution today's animal producers and feed companies are paying attention not only on optimal productivity but also minimizing N pollution, carbon footprint and ammonia emissions. Furthermore, to feed the increasing world population, e.g. from 7 billion in 2014 to 9 billion people by 2050, we need to produce more food including meat whereas the possibility of increasing land use area will be more and more limited in most countries. Replacing a portion of AA contributed by natural protein sources with supplemental AA greatly reduces the amount of crop resources needed per animal, and as a result allows to feed more animals with same crop production.

However, as the body cannot store AA for future use, it should not be overlooked that body protein synthesis is an "all or nothing" event. Pigs will stop growing even if a single AA is deficient in the diet. In general, lysine (Lys) is the first limiting AA followed by threonine (Thr), methionine (Met), tryptophan (Trp), Val and isoleucine (Ile) in typical low CP pig diets. In theory, the performance of the pigs should be maintained if all AA levels are adequately balanced in the low CP diets. Formulating diets on the basis of the standardized ileal digestible (SID) AA in combination with the ideal protein concept and net energy (NE) allow lowering CP level without affecting the performance of starter, grower and finisher pigs. However, when the dietary CP level was reduced more than 4 to 6 %-points, one or more nonessential amino acids (NEAA) and/or essential amino acids (EAA) (beyond the first 5 limiting AA) can become limiting. If this is ignored, as it happened sometimes, pig performance will likely be reduced even though diets are balanced on ileal digestible AA and NE basis.

For the successful implementation of low CP, AA-fortified diets at the production level, growth performance of pigs fed the low CP-AA diets as well as the price of low CP diets should be comparable to that of high CP diets. For developing low CP diets which assure for optimal performance, a great amount of research has been done looking at the effects of reducing dietary CP level and balancing with AA on performance of pigs over the last two decades.

Thus, this review paper will focus 1) to review the literature data and provide an update on the effects of lowering the dietary CP level on performance, N retention and N excretion of starter, grower and finisher pigs, 2) to discuss the potential reasons for reduced pig performance associated with low CP diets, and 2) to address the possibility of using low CP, AA-supplemented diets for pigs.

Because the CP content and potential for reducing dietary CP level are greater in starter pigs compared with older pigs, this review will be split into two parts to allow more focus. The first part focuses on the effects of reduced CP diets on performance of starter pigs [final body weight (BW) less than 30 kg] while the second and third parts focus on growing-finishing pigs (final BW greater than 30 kg) and the effects of low CP diets on N balance. For a better overview the studies without and with detrimental effects of low CP diets are listed separately within each group. The low CP treatments reported in Tables 1, 2 and 3 were the lowest or one of the lowest CP treatments reported by each research group.

EFFECTS OF FEEDING LOW CP, AMINO ACID-SUPPLEMENTED DIETS ON THE PERFORMANCE OF STARTER PIGS

A review of the performance such as average daily gain (ADG) and feed conversion ratio (FCR) of starter pigs fed low CP diets relative to their high CP counterparts and dietary nutrient levels (SID Lys, SID Lys:CP, energy) is given in Table 1.

Studies without detrimental effects of performance by reducing dietary CP content

The ADG and FCR of 6 to 8 kg weaned pigs were not affected while the fecal consistency (diarrhea incidence) score was improved by 7 %-points CP reduction when balanced for the same level of EAA on SID basis relative to the high CP diets (Heo *et al.*, 2008, 2009). More recently, Girotto Junior *et al.* (2013) and Toledo *et al.* (2014) found that ADG or FCR of 6 to 14 kg pigs was practically not affected by lowering the dietary CP level by 5 to 6 %-points when all EAA are well balanced on SID basis. Similar positive results of low CP diets for 7 to 13 kg pigs were also reported (Heo *et al.*, 2010; Nemechek *et al.*, 2014). By balancing similar levels of EAA on SID basis and NE relative to the high CP diets, 4 to 6 %-points CP reduction was possible without affecting the performance of 8 to 17 kg pigs

TABLE 1 Effects of reducing CP and supplementing with amino acids on performance of starter pigs[1]

BW	DIETARY CP %		ENERGY	SID LYS	SID LYS: CP	AA added to LP diet	HP	LP performance (relative to HP)		DIETARY AA in LP diet	REFERENCE
kg	HP	LP	MJ/kg	%	%		ADG g	ADG g	FCR		
Studies without detrimental effects of performance by reducing dietary CP content											
6–8	24.3	17.3	13.8 DE	1.10	6.4	Lys, Thr, Met, Trp, Val, Ile	148	-5	-0.09	Adequate	Heo *et al.*, 2008[++]
6–8	24.0	17.5	13.8 DE	1.08	6.2	Lys, Thr, Met, Trp, Val, Ile	129	+21	+0.08	Adequate	Heo *et al.*, 2009[++]
6–14	24.0	19.0	14.4 ME	1.40	7.4	Lys, Thr, Met, Trp, Val, Ile	368	-7	+0.16	Adequate	Girotto Junior *et al.*, 2013[7]
6–16	21.0	15.0	13.3 ME	1.33	8.9	Lys, Thr, Met, Trp, Val, Ile	348	+1	0.00	Adequate	Toledo *et al.*, 2014[5]
7–10	23.9	19.0	14.1 DE	1.13	5.9	Lys, Thr, Met, Trp, Val, Ile	214	+14		Adequate	Heo *et al.*, 2010[7, ++]
7–12	21.1	20.3	14.0 ME	1.30	6.4	Lys, Thr, Met, Trp, Val	376	+4	+0.03	Adequate	Nemechek *et al.*, 2014
7–13	21.1	19.4	14.0 ME	1.30	6.7	Lys, Thr, Met, Trp, Val	347	+40	0.00	Adequate	Nemechek *et al.*, 2014
		18.9	14.0 ME	1.30	6.9	Lys, Thr, Met, Trp, Val		-11	+0.09	Adequate	
8–17	23.9	20.0	10.7 NE	1.30	6.5	Lys, Thr, Met, Trp	429	-19	+0.01	Adequate	Htoo *et al.*, 2007[+]
12–26	22.4	16.9	10.5 NE	1.07	6.3	Lys, Thr, Met, Trp Val, Ile	642	+21	+0.08	Adequate	Le Bellego and Noblet, 2002[6]
13–27	19.7	16.6	14.6 DE	1.14	6.9	Lys, Thr, Met, Trp	590	-19	+0.12	Adequate	Montalvo *et al.*, 2013
14–28	18.0	15.0	14.1 ME	0.95[2]	6.3[3]	Lys, Thr, Met, Trp	646	+4	+0.02	Adequate	Jin *et al.*, 1998
Studies with detrimental effects of performance by reducing dietary CP content											
6–12	21.3	18.7	10.7 NE	1.35	7.2	Lys, Thr, Met, Trp, Ile	266	-29[4]	+0.18[4]	Def. in Val, His and Phe	Opapeju *et al.*, 2008[7]
		17.0	10.7 NE	1.35	8.0	Lys, Thr, Met, Trp, Ile, Val		-26[4]	+0.26[4]	Def. in Leu, His and Phe	
6–14	22.8	18.9	14.6 ME	1.40	7.4	Lys, Thr, Met, Trp	353	-65[4]	+0.12[4]	Def. in Val, His and Phe	Nyachoti *et al.*, 2006[8]
		17.4	14.6 ME	1.40	8.0	Lys, Thr, Met, Trp, Ile	353	-121[4]	+0.29[4]	Def. in Val, His, Phe and Leu	
7–10	23.1	18.9	13.9 ME	1.30	6.9	Lys, Thr, Met, Trp	266	-14	+0.02	Adequate in EAA	Yue and Qiao, 2008[9]
		17.2	13.9 ME	1.30	7.6	Lys, Thr, Met, Trp, Ile, Val, His, Phe	266	-57[4]	+0.13[4]	Def. in NEAA (> 30 % lower in Gln, Glu, Gln)	Nemechek *et al.*, 2014
8–12	20.7	16.7	14.4 DE	1.07	6.4[3]	Lys, Thr, Met, Trp, Ile, Val, Leu	299	-23	+0.16[4]	Likely def. in His and Ser	Deng *et al.*, 2009
		12.7	14.4 DE	1.07	8.4[3]	Lys, Thr, Met, Trp, Ile, Val, Leu		-35[4]	+0.28[4]	Likely def. in His, Ser, Arg, Gln, Gly	
8–16	20.0	18.0	13.5 DE	1.25[2]	6.9[3]	Lys, Thr, Met, Trp	294	-21[4]	+0.05	Likely def. in Val and Ile	Li *et al.*, 1998
12–26	17.4	13.4	10.3 NE	0.92	6.9	Lys, Thr, Met, Trp, Ile, Val, His, Leu, Phe	450	-8	+0.06	Adequate	Gloaguen *et al.*, 2014[5]
		11.6	10.3 NE	0.89	7.7	Lys, Thr, Met, Trp, Ile, Val, His, Leu, Phe		-92[4]	+0.35[4]	Def. in NEAA	
		12.5	10.6 NE	0.92	7.4	Lys, Thr, Met, Trp, Ile, Val, His, Leu, Phe, Arg, Glu, Gly, Pro		-30	+0.23[4]	Def. in NEAA	
		13.4	10.8 NE	0.93	6.9	Lys, Thr, Met, Trp, Ile, Val, His, Leu, Phe, Arg, Glu, Gly, Pro		+1	+0.03	Adequate	

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[1] Abbreviations: DE = Digestible energy; ME = Metabolizable energy; NE = Net energy; HP = High protein diet; LP = Low protein diet; Def. = deficient

[2] Total Lys content

[3] Total Lys:CP ratios

[4] Significantly different from HP diet ($P < 0.05$)

[5] Similar level of electrolyte balance (mEq/kg) was balanced in both high and low CP diets

[6] Dietary electrolyte balance was lower (−79 mEq/kg) in low CP diet (vs. high CP diet)

[7] Dietary CP reduction did not affect villus height in duodenum, jejunum and ileum

[8] Dietary CP reduction did not affect villus height in duodenum and in ileum but reduced villus height in jejunum

[9] Dietary CP reduction from 23.1 to 17.2 % had no effect on gut morphology but lowering to 17.2 % decreased villus height in duodenum and in jejunum and lactase and sucrase activities in jejunum

[+] Ileal concentration of ammonia and amines reduced by lowing dietary CP level

[++] Fecal consistency (diarrhea incidence) was improved by lowing dietary CP level

(Htoo *et al.*, 2007) and 12 to 26 kg pigs (Le Bellego and Noblet, 2002). Performance of 13 to 27 kg pigs (Montalvo *et al.*, 2013) and 14 to 28 kg pigs (Jin *et al.*, 1998) were not affected by reducing the dietary CP level by 3 %-points when EAA were adequately balanced. It should be mentioned that the highest dietary SID Lys:CP ratio in these studies (except Girotto Junior *et al.*, 2013 and Toledo *et al.*, 2014) was 6.9 % (7.5 % total Lys:CP).

Studies with detrimental effects of performance by reducing dietary CP content

Opapeju *et al.* (2008) reported that performance (ADG, FCR) of 6 to 12 kg pigs was impaired when dietary CP was reduced from 21.3 to 18.7 or 17.0 % and balanced for the first 5 or 6 limiting AA. It became clear that some EAA [Val, histidine (His), leucine (Leu), phenylalanine (Phe)] were limiting in the low CP diets. Similar reduction in performance of 6 to 12 kg pigs was found when some EAA (Val, His, Leu, Phe) were ignored when balancing AA contents in low CP diets (Nyachoti *et al.*, 2006). Yue and Qiao (2008) reported that performance of 7 to 10 kg pigs was not affected at 4 %-points CP reduction when diets were balanced for EAA on SID basis but a decline in performance, villus height in duodenum and in jejunum and lactase and sucrase activities in jejunum were observed with 6 %-points CP reduction (18.9 to 17.2 % CP). The SID Lys:CP of 7.6 % and analyzed AA results indicate that 17.2 % CP diet contained insufficient level of NEAA [glutamine (Gln) and glutamate (Glu)] or N to synthesis NEAA. Deng *et al.* (2009) found that performance of 8 to 12 kg pigs was reduced when dietary CP was lowered from 20.7 to 16.7 or 12.7 % even though the diets were balanced for 7 EAA on SID basis. Based on plasma AA levels, one or more AA [arginine (Arg), Glu, glycine (Gly), His, serine (Ser)] may be limiting in the low CP diets. Considering only the first 4 limiting AA (ignoring Val, Ile) in the low CP diet was not sufficient to maintain ADG of 8 to 16 pigs (Li *et al.*, 1998). Recently, Gloaguen *et al.* (2014) showed that lowering CP at 4 %-points (17.4 to 13.4 %) in cereals-soybean meal based diets while balancing for adequate levels of all EAA and similar level of NE and electrolyte balance had no effect on ADG and FCR of 12 to 22 kg pigs but lowering up to 11.6 % reduced pig performance due to insufficient level of NEAA as indicated by 7.7 % SID Lys:CP. Pigs fed 13.4 % CP cereals-based diet supplemented with all 10 EAA and NEAA [Glu, Gly and proline (Pro)] having 7.4 % SID Lys:CP had a poorer FCR but a greater level of addition with Arg, Glu, Gly and Pro to achieve at 6.9 % SID Lys:CP (13.4 % CP) recovered the ADG and FCR similar to that of 17.4 % CP diet. These results indicate that not only EAA but also NEAA should be considered particularly for extremely low CP diets.

EFFECTS OF FEEDING LOW CP, AMINO ACID-SUPPLEMENTED DIETS ON THE PERFORMANCE OF GROWING-FINISHING PIGS

A review of the performance (ADG and FCR) of growing-finishing pigs fed low CP diets relative to their high CP counterparts and dietary nutrient levels (SID Lys, SID Lys:CP, energy) is shown in Table 2.

TABLE 2 Effects of reducing CP and supplementing with amino acids on performance of growing-finishing pigs[1]

BW	DIETARY CP %		ENERGY	SID LYS	SID LYS: CP	AA added to LP diet	HP	LP performance (relative to HP)[1]			DIETARY AA in LP diet	REFERENCE
kg	HP	LP	MJ/kg	%	%		ADG g	FCR	Dres. %	BF cm		
Studies reported without detrimental effects of performance by reducing dietary CP content												
9-93	19.0	15.0	n.a.	1.04[2]	6.9[3]	Lys, Thr, Trp	+10	+0.07	+1.00[4]	+0.08	Adequate	Kerr *et al.*, 1995
(3 phases)	16.0	12.0	n.a.	0.82[2]	6.8[3]	Lys, Thr, Trp						
	14.0	11.0	n.a.	0.67[2]	6.1[3]	Lys, Thr, Trp						
19-39	18.2	13.4	13.6 NE	0.83	6.2	Lys, Thr, Met, Trp, Val, Ile	−27	+0.12			Adequate	Powell *et al.*, 2011
21-40	18.2	13.4	13.6 NE	0.83	6.2	Lys, Thr, Met, Trp, Val, Ile, Gly	+9	+0.09			Adequate	
23-44	18.2	13.4	13.6 NE	0.83	6.2	Lys, Thr, Met, Trp, Val, Ile, Gly, Arg	−13	−0.02			Adequate	
		13.4	13.6 NE	0.83	6.2	Lys, Thr, Met, Trp, Val, Ile, Gly, Glu	−8	−0.02			Adequate	
20-32	16.0	14.0	13.6 DE	0.75[2]	5.4[3]	Lys, Thr, Met, Trp	14	−0.15			Adequate	Li *et al.*, 1998
30-48	16.0	14.0	13.6 DE	0.75[2]	5.4[3]	Lys, Thr, Met, Trp	114	−0.20				
50-72	14.0	12.0	12.8 DE	0.63[2]	5.3[3]	Lys, Thr, Met, Trp	197	−0.71[4]				
20-55	16.6	13.0	14.2 DE	0.66	5.1	Lys, Thr, Trp, Val, Ile	−10	+0.08		+0.50	Adequate	Tuitoek *et al.*, 1997
55-100	14.2	11.0	14.2 DE	0.55	5.0	Lys, Thr, Trp, Val, Ile						
20-46	16.3	14.0	9.9 NE	0.75[2]	5.3[3]	Lys, Thr, Met, Trp,	+76	+0.05		+0.18	Adequate	Figueroa *et al.*, 2002
		12.2	9.9 NE	0.76[2]	6.2[3]	Lys, Thr, Met, Trp,	46	−0.03		+0.33		
23-37 **23 vs. 33 °C**	16.2	13.0	13.6 ME	0.84[2]	6.5[3]	Lys, Thr, Trp	−44 +8	+0.15 −0.11			Adequate	Kerr *et al.*, 2003a
23-53	19.0	14.4	9.9 NE	0.83	5.8	Lys, Thr, Met, Trp	+10	0.00			Adequate	Yi *et al.*, 2010
28-52	19.0	14.6	9.8 NE	0.88	6.0	Lys, Thr, Met, Trp	+10	−0.04				
23-60	16.0	14.0	13. 5 ME	0.83	6.0	Lys, Thr, Met, Trp	−10	+0.05			Adequate	Madrid *et al.*, 2013
60-95	15.5	13.5	13. 5 ME	0.74	5.3	Lys, Thr, Met, Trp	−30	+0.11				
25-110	20.4	17.0	10.2 NE	1.00	5.9	Lys, Thr, Met, Trp, Val, Ile, His, Phe, Arg, Gly, Glu, Pro,	−30	−0.09	+0.40	+0.13	Adequate	Htoo *et al.*, 2013
(4 phases)	20.2	16.4	10.0 NE	0.86	5.3	Lys, Thr, Met, Trp, Val, Ile, Arg, Gly, Glu, Pro,						
	17.3	13.9	9.8 NE	0.74	5.3	Lys, Thr, Met, Trp, Val, Ile						
	16.1	12.4	9.6 NE	0.64	5.2	Lys, Thr, Met, Trp, Val, Ile						
27–100	20.1	15.6	10.3 NE	0.88	5.6	Lys, Thr, Met, Trp, Val, Ile	−20	−0.16[4]	+0.80	−0.03	Adequate	Le Bellego *et al.*, 2002[5]‡
(2 phases)	17.5	13.3	10.4 NE	0.73	5.5	Lys, Thr, Met, Trp, Val, Ile						
27-111	15.5	13.9	9.7 NE	0.78	5.6	Lys, Thr, Met, Trp, Val	+17	−0.07			Adequate	Quiniou *et al.*, 2011
28-117	15.3	11.3	n.a.	0.95[2]	6.8[3]	Lys, Thr, Met, Trp, Val, Ile	-40	+0.08		+0.31	Adequate	Shriver *et al.*, 2003
29-51	16.8	12.9	13.8 ME	0.84[2]	6.5[3]	Lys, Thr, Met	+13	−0.04			Adequate	Kerr *et al.*, 2003b
30-70	20.0	14.0	14.0 DE	1.00[2]	7.1[3]	Lys, Thr, Met, Trp	+2	−0.01			Adequate	Pieterse *et al.*, 2000
30-60	17.0	13.0	14.2 DE	0.79	6.0	Lys, Thr, Met	-31	+0.19			Adequate	Ferreira *et al.*, 2007
55-102	15.9	13.6	8.1 NE	0.81	6.0	Lys, Thr, Met, Trp, Val	+11	+0.06			Adequate	Hansen *et al.*, 2014
60-100	17.3	12.1	14.2 DE	0.77	6.3	Lys, Thr, Met, Trp, Val, Ile	-72	+0.14			Adequate	Orlando *et al.*, 2007
69-95	16.0	12.0	10.5 NE	0.67	5.6	Lys, Thr, Met, Trp	-40	+0.16	+1.10	+0.80	Adequate	Chen *et al.*, 2011
		11.2	10.1 NE	0.66	5.9	Lys, Thr, Met, Trp	+10	+0.00	−0.10	0.00		
		11.9	9.9 NE	0.66	5.5	Lys, Thr, Met, Trp	+10	+0.04	+0.10	−0.60		
68-95 **19 vs. 31 °C**	16.2	13.7	13.6 ME	0.75	5.4	Lys, Thr, Met, Trp	+11 +107	+0.07 −0.07	+0.00 +0.40	+0.48[4] +0.24[4]	Adequate	Rodrigues *et al.*, 2012[5]
70-100	18.0	13.5	13.5 ME	0.81	6.0	Lys, Thr, Met, Trp, Val	−36	−0.01	+0.93	−0.46	Adequate	Vidal *et al.*, 2010
83-173	12.0	9.8	13.2 ME	0.65[2]	6.6[3]	Lys, Thr, Met, Trp	21	+0.03	+0.60	+0.30	Adequate	Galassi *et al.*, 2010

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[1] Abbreviations: n.a. = not available; DE = Digestible energy; GE = Gross energy; ME = Metabolizable energy; NE = Net energy; HP = High protein diet; LP = Low protein diet; Dres. = Dressing percentage; BF = Back fat; Def. = deficient

[2] Total Lys content

[3] Total Lys:CP ratios

[4] Significantly different from HP diet ($P<0.05$)

[5] Similar level of electrolyte balance (mEq/kg) was balanced in both high and low CP diets

TABLE 2 Effects of reducing CP and supplementing with amino acids on performance of growing-finishing pigs[1] **(continued)**

BW	DIETARY CP %		ENERGY	SID LYS	SID LYS: CP	AA added to LP diet	HP	LP performance (relative to HP)[1]			DIETARY AA in LP diet	REFERENCE
kg	HP	LP	MJ/kg	%	%		ADG g	FCR	Dres. %	BF cm		
Studies reported without detrimental effects of performance by reducing dietary CP content												
75-120	12.6	9.1	11.3 NE	0.64[2]	7.0[3]	Lys, Thr, Met, Trp, Val	0	−0.15	−1.30		Adequate	Knowles *et al.*, 1998
77-108	15.2	12.1	11.1 NE	0.75[2]	6.2[3]	Lys, Thr, Met, Trp, Val, Ile	−50	+0.12	+0.20			
85-119	13.4	9.7	13.9 ME	0.54	5.6	Lys, Thr, Trp	−50	+0.18	+1.70		Adequate	Dean *et al.*, 2007
	13.3	9.5	13.8 ME	0.54	5.7	Lys, Thr, Trp	6.1	−0.26	+1.20			
Studies reported detrimental effects of performance by reducing dietary CP content												
25-41	20.2	16.2	10.6 NE	0.96	5.9	Lys, Thr, Met, Trp	−3	+0.17			Adequate	Kerr *et al.*, 2003b
	21.0	17.7	10.4 NE	0.96	5.4	Lys, Thr, Met, Trp	−4	+0.14			Adequate	
	20.9	17.9	10.1 NE	0.96	5.4	Lys, Thr, Met, Trp	−68[4]	+0.34[4]			NE content was limiting	
32-90	16.2	12.0	16.5 GE	0.64	5.4	Lys, Thr, Met, Trp	−90[4]	+0.13[4]			Lower Lys relative to HP	Gomez *et al.*, 2002
(2 phases)	14.2	10.2	16.6 GE	0.56	5.5	Lys, Thr, Met, Trp					Def. in Thr and Trp	
37-60	16.1	12.8	9.5 NE	0.62	4.8	Lys, Thr, Trp	−101[4]	+0.23[4]			Def. in Thr.	Guay *et al.*, 2006
		10.1	9.6 NE	0.62	6.1	Lys, Thr, Met, Trp, Val, Ile, Phe	−171[4]	+0.44[4]			Def. in Thr and Lys	
		7.8	9.6 NE	0.62	7.9	Lys, Thr, Met, Trp, Val, Ile, Phe, His, Leu, Arg	−313[4]	+0.97[4]			Def. in Thr, Lys, NEAA	
45-96	20.8	15.0	14.4 DE	1.13[2]	7.5[3]	Lys, Thr, Met, Trp	+86	−0.11[4]	+0.24	+1.22[4]	Balanced for first 4 AA	Carpenter *et al.*, 2004
		13.3	10.4 NE	0.73	5.5	Lys, Thr, Met, Trp, Val, Ile						
55-105	16.0	13.0	13.8 ME	0.92[2]	7.1[3]	Lys, Thr, Met, Trp	−60[4]	+0.11	+2.28[4]	+0.21	Likely deficient in Val and Ile	Lee *et al.*, 2001
(3 phases)	14.0	11.0	13.8 ME	0.80[2]	7.3[3]	Lys, Thr, Met, Trp						
	12.0	9.0	13.8 ME	0.67[2]	7.4[3]	Lys, Thr, Met, Trp						
56-114	14.3	12.8	13.6 ME	0.70[2]	5.5[3]	Lys	−128[4]	+0.72[4]	−1.00		Def. in Thr and Met+Cys	De La Llata *et al.*, 2002
(2 phases)	13.2	10.4	13.6 ME	0.55[2]	5.3[3]	Lys					Def. in Val and Lys	
85-121	13.4	8.5	13.8 ME	0.54	6.3	Lys, Thr, Met, Trp	−220	+0.68	−2.94[4]	−0.01	Def. in Thr and Met+Cys	Dean *et al.*, 2007

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[1] Abbreviations: n.a. = not available; DE = Digestible energy; GE = Gross energy; ME = Metabolizable energy; NE = Net energy; HP = High protein diet; LP = Low protein diet; Dres. = Dressing percentage; BF = Back fat; Def. = deficient

[2] Total Lys content

[3] Total Lys:CP ratios

[4] Significantly different from HP diet ($P < 0.05$)

Studies without detrimental effects of performance by reducing dietary CP content

Kerr *et al.* (1995) demonstrated that reducing 4 %-points reduction (3 phases) while balancing to meet the requirement of the first 4 AA did not affect the performance and backfat thickness but increased carcass dressing percentage of 9 to 93 kg pigs. In a series of studies, Powell *et al.* (2011) showed that 5 %-points CP reduction (18.2 to 13.4 %) and supplementing with EAA and NEAA (Gly and Glu) had no effect on performance of growing pigs with BW ranged from 19 to 44 kg. Similarly, Li *et al.* (1998) conducted a series of trials and reported that 2 %-points CP reduction had no effect on performance of growing pigs with BW ranged from 20 to 72 kg. Even FCR was improved by lowering dietary CP from 14 to with 12 % which agrees with Le Bellego *et al.* (2002) who also observed an improved FCR of 27 to 100 pigs fed the low CP diet. Improved FCR was likely due to a more efficient AA utilization.

When all EAA and energy are adequately balanced, there was no difference in ADG, FCR, dressing percentage or backfat thickness of pigs with a wide BW range from 20 to 173 kg fed low CP diets relative to those fed typical high CP diets (Tuitoek *et al.*, 1997; Figueroa *et al.*, 2002; Htoo *et al.*, 2013; Shriver *et al.*, 2003; Chen *et al.*, 2011; Vidal *et al.*, 2010; Galassi *et al.*, 2010; Knowles *et al.*, 1998; Dean *et al.*, 2007). Numerous other studies also have consistently proven that the ADG and FCR of growing-finishing pigs with BW ranged from 23 to 111 kg were practically not affected by 2 to 6 %-points CP reduction provided that the diets were adequately balanced for all EAA and energy (Yi *et al.*, 2010; Madrid *et al.*, 2013; Quiniou *et al.*, 2011; Kerr *et al.*, 2003b; Pieterse *et al.*, 2000; Ferreira *et al.*, 2007; Hansen *et al.*, 2014; Orlando *et al.*, 2007). Kerr *et al.* (2003a) evaluated the effect of reducing dietary CP from 16.2 to 13.0 % under two climatic conditions (23 vs. 33 °C) in 23 to 37 kg growing pigs. The ADG and FCR were not affected by low CP diet under both climatic conditions but the FCR was numerically improved by feeding low CP diet under hot climate. Similarly, a slight improvement in FCR of 68 to 95 kg pigs by reducing dietary CP from 16.2 to 13.7 % was reported (Rodrigues *et al.*, 2012). However, they found an increase in backfat thickness of pigs fed by 13.7 % CP diet which may be due to more efficient energy utilization associated with low CP diet as a portion of energy retained can be deposited as body fat.

Studies with detrimental effects of performance by reducing dietary CP content

Kerr *et al.* (2003b) reported that performance (ADG, FCR) of 29 to 51 kg pigs was not affected by 4 %-points CP reduction when the NE content was maintained at the same (10.6 MJ/kg) or slightly lower (10.4 MJ/kg) level relative to that of high CP diet but performance was reduced when the NE was reduced to 10.1 MJ/kg in the low CP diet, indicating that NE in low CP diet should not be undersupplied. Gomez *et al.* (2002) found a decline in the performance of 32 to 90 kg pigs when the dietary CP was reduced at 4 %-points. A closer look at the dietary AA contents indicated that Lys was lower in the low CP grower diet, and Thr and Trp were deficient in the low CP finisher diet. Similarly, Guay *et al.* (2006) reported that the ADG and FCR of 37 to 60 kg pigs fed twice daily were impaired by 3, 6 or 8 %-points CP reductions compared with pigs fed normal CP (16 %) diet despite the fact that diets were balanced to meet SID requirement for EAA and similar NE content. The analyzed AA values revealed that the low CP diets were deficient in one or more AA (Thr, Lys and some NEAA) which may have reduced performance.

Carpenter *et al.* (2004) reported that performance of 45 to 96 kg pigs was not affected when the dietary CP was reduced from 21 to 15 % but FCR and backfat content were affected when dietary CP was reduced to 12 % while balancing for the first 4 AA and the same digestible energy (DE) content. The Lys:CP was 9.1 % in the 12 % CP diet indicate that NEAA or N for NEAA synthesis was deficient. A greater backfat thickness associated with CP reduction may be due to more efficient energy utilization of pigs fed low CP diet. Some studies found detrimental effects on ADG, FCR or carcass dressing percentage of pigs with BW ranged from 55 to 121 kg when dietary CP was reduced 2 to 5 %-points (Lee *et al.*, 2001; De La Llata *et al.*, 2002 and Dean *et al.*, 2007). A closer look at the dietary AA contents indicated that one or more EAA were limiting which negatively affected pig performance in these studies (Table 2).

EFFECTS OF FEEDING LOW CP, AMINO ACID-SUPPLEMENTED DIETS ON NITROGEN BALANCE OF PIGS

In addition to the growth performance trials, N-balance assays were also conducted to study the effect of CP reduction on the retention and excretion of N from swine production. A review of the N balance and N excretion of pigs fed low CP diets relative to their high CP diets and dietary nutrient levels (SID Lys, SID Lys:CP, energy) are shown in Table 3.

TABLE 3 Effects of reducing CP and supplementing with amino acids on N balance of pigs[1]

BW	DIETARY CP %		ENERGY	SID LYS	SID LYS: CP	AA added to LP diet	HP	LP performance (relative to HP)			DIETARY AA in LP diet	REFERENCE
kg	HP	LP	MJ/kg	%	%		N exc. g/d	N retain. g/d	N retain. %	N exc. %[2]		
Studies reported without detrimental effects of retained N by reducing dietary CP content												
11	21.0	15.0	13.3 ME	1.33	8.9	Lys, Thr, Met, Trp, Val, Ile	−3.0[5]	−3.9	+6.5[5]	−7.9	Adequate	Toledo *et al.*, 2014[6]
15	22.4	16.9	10.5 NE	1.07	6.3	Lys, Thr, Met, Trp, Val, Ile	−5.6[5]	−2.2	+12.9[5]	−9.5	Adequate	Bellego and Noblet, 2002[7],[8]
50	17.2	14.3	13.5 ME	0.83	5.8	Lys, Thr, Met, Trp	−7.2[5]	+0.2	+5.1[5]	−9.3	Adequate	Hernandez *et al.*, 2011
88	15.5	13.5	13.5 ME	0.74	5.5	Lys, Thr, Met, Trp	−4.8	+0.1	+3.1[5]	−9.1	Adequate	
53	20.8	15.0	14.4 DE	1.13[3]	7.5[4]	Lys, Thr, Met, Trp	−12.0[5]		+8.0[5]	−5.8	Adequate	Carpenter *et al.*, 2004
		12.3	14.1 DE	1.12[3]	9.1[4]	Lys, Thr, Met, Trp	−13.2[5]		+5.0[5]	−4.3	Adequate	
55	16.5	12.5	9.4 NE	0.82	6.6	Lys, Thr, Met, Trp	−13.4[5]	−7.5	+8.8[5]	−8.8	Adequate	Canh *et al.*, 1998
64	17.4	13.9	10.3 NE	0.87	6.3	Lys, Thr, Met, Trp, Val, Ile	−12.7[5]	−3.0	+9.3[5]	−10.9	Adequate	Noblet *et al.*, 2001
		14.9	11.0 NE	0.91	6.1	Lys, Thr, Met, Trp, Val, Ile	−11.7[5]	−2.1	+9.0[5]	−14.1	Adequate	
65	18.9	14.6	10.4 NE	0.89	6.1	Lys, Thr, Met, Trp, Val, Ile	−12.2[5]	−2.9	+7.7[5]	−7.7	Adequate	Le Bellego *et al.*, 2001
		12.3	10.5 NE	0.89	7.2	Lys, Thr, Met, Trp, Val, Ile	−21.3[5]	−3.7	+18.0[5]	−8.7	Adequate	
65	17.4	13.9	10.3 NE	0.87	6.3	Lys, Thr, Met, Trp, Val, Ile	−13.0[5]	−1.2	+11.0[5]	−10.0	Adequate	
74	20.3	15.2	13.7 DE	0.99[3]	6.5[4]	Lys, Thr, Met	−16.0[5]	+1.1	+10.0[5]	−7.1	Adequate	Lynch *et al.*, 2007[7]
74	19.8	15.4	13.6 DE	0.99[3]	6.4[4]	Lys, Thr, Met	−17.6[5]	+5.4	+22.0[5]	−10.7	Adequate	Lynch *et al.*, 2008
	20.2	14.6	13.6 DE	0.99[3]	6.8[4]	Lys, Thr, Met	−11.8[5]	−5.2	+5.4[5]	−6.6	Adequate	
80	20.6	13.2	9.78 NE	1.11[3]	8.4[4]	Lys, Thr, Met, Trp	−33.8[5]	+5.0	+28.8[5]	−8.7	Adequate	Leek *et al.*, 2005,[9]
152	12.0	9.8	13.2 ME	0.65[3]	6.6[4]	Lys, Thr, Met, Trp	−6.0[5]	−3.1	+0.9	−8.1	Adequate	Galassi *et al.*, 2010
Studies reported detrimental effects of retained N by reducing dietary CP content												
10	19.4	16.6	10.2 NE	1.16	7.0	Lys, Thr, Met, Trp, Val	−1.6[5]	−0.1	+9.4[5]	−10.2	Adequate	Gloaguen *et al.*, 2014
		14.0	10.5 NE	1.17	8.4	Lys, Thr, Met, Trp, Ile, Val, His, Leu, Phe	−2.4[5]	−1.6[5]	+11.2[5]	−7.9	Deficient in NEAA	
22	15.7	11.8	14.4 ME	0.83[3]	6.9[4]	Lys, Thr, Trp	−4.1[5]	−18.7[5]	+7.0[5]	−9.4	Deficient in NEAA	Kerr and Easter, 1995
		15.6	14.7 ME	0.98[3]	5.2[4]	Lys, Thr, Trp, Gly, Glu	+0.5	−0.6	−2.0		Adequate	
36	18.2	14.5	14.1 DE	0.83	5.7	Lys, Thr, Met	−5.4[5]	−2.3[5]	+5.8[5]	−7.9	Marginally limiting in Trp, Met+Cys.	Deng *et al.*, 2007
		13.6	14.1 DE	0.83	6.1	Lys, Thr, Met	−6.6[5]	−3.5[5]	+6.3[5]	−7.6	Def. in Val, Ile, Trp, Met+Cys	
41	18.6	12.1	10.0 NE	0.72[3]	5.9[4]	Lys, Thr, Met, Trp,	−5.8[5]	−7.9[5]	+2.6	−5.2	Lower levels of Lys (−0.28 %) and other AA	Figueroa *et al.*, 2002
50	14.6	11.0	14.3 ME	0.68[3]	6.2[4]	Lys, Thr, Met, Trp, Val, Ile, Glu	−6.9[5]	−2.2[5]	+8.8[5]	−10.2	Likely deficient in NEAA (– 30 % in Gly).	Otto *et al.*, 2003
		9.9	14.2 ME	0.65[3]	6.6[4]	Lys, Thr, Met, Trp, Val, Ile, His, Phe, Leu, Glu	−11.9[5]	−4.3[5]	+17.9[5]	−13.2	Likely deficient in NEAA (– 40 % in Gly)	

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[1] Abbreviations: DE = Digestible energy; GE = Gross energy; ME = Metabolizable energy; NE = Net energy; HP = High protein diet; LP = Low protein diet; N exe. = N excretion; N retain. = N retained; Def. = deficient

[2] Reduction in N excretion (%/1 %-point CP reduction)

[3] Total Lys content

[4] Total Lys:CP ratios

[5] Significantly different from HP diet ($P<0.05$)

[6] Similar level of electrolyte balance (mEq/kg) was balanced in both high and low CP diets

[7] Dietary electrolyte balance was lower (−166 mEq/kg) in low CP diet (vs. high CP diet)

[8] Pigs fed low CP diet consumed less water (−296 g/d) and excreted less urine (−276 g/d) relative to the high CP diet

[9] Pigs fed low CP diet excreted less urine (−4.05 L/d), less manure (−3.71 kg/d) and less ammonia N in manure (−0.72 g/d)

Studies without detrimental effects of nitrogen retention by reducing dietary CP content

By balancing to meet optimal SID levels of all EAA and dietary energy, it is possible to reduce the dietary CP level by 6 %-points without affecting N retained [g/day (d)] of 11 and 15 kg starter pigs (Toledo *et al.*, 2014; Bellego and Noblet, 2002). A larger body of research (15 datasets) with growing-finishing pigs with BW ranged from 50 to 152 kg demonstrated that reducing the dietary CP levels ranging from 2 to 9 %-points did not affect the N retained (g/d) while improving N retention (%) provided that diets are well balanced for EAA and adequate energy (Hernandez *et al.*, 2011; Carpenter *et al.*, 2004; Canh *et al.*, 1998; Noblet *et al.*, 2001; Le Bellego *et al.*, 2001; Lynch *et al.*, 2007; Lynch *et al.*, 2008; Leek *et al.*, 2005; Galassi *et al.*, 2010).

All the N balance data consistently reported a significant decrease in total N excretion while N-retention (% of N intake) improved considerably. A majority of the diets used in these studies were balanced on SID amino acids and NE basis. It should be noted that lowering CP in the diets, i.e. replacing a part of SBM with crystalline AA, typically resulted to a reduced dietary electrolyte balance, calculated as sodium (Na) + potassium (K) – chloride (Cl) according to Patience and Chaplin (1997), e.g. 70 to 166 mEq/kg less in the low CP diets reported by Bellego and Noblet (2002), Lynch *et al.* (2007) and Leek *et al.* (2005). However, it did not affect the N retention of the pigs which agrees with Patience and Chaplin (1997) who showed that the performance of 35 to 41 kg pigs was not affected even the diet has a negative electrolyte balance (–20 mEq).

Studies with detrimental effects of nitrogen retention by reducing dietary CP content

Nitrogen retained (g/d) was not affected when dietary CP was reduced from 19.4 to 16.6 % in 10 kg piglets but lowering the CP level further to 14 % (SID Lys:CP of 8.4 %) reduced the retained N which was likely attributed to some of NEAA became limiting (Gloaguen *et al.*, 2014). Kerr and Easter (1995) found that N retained (g/d) in 22 kg pigs was reduced when dietary CP was reduced from 16 to 12 % while balancing for EAA on total basis. Interestingly, N retained (g/d) was not different from that of high CP diet when L-Gly and L-Glu were added additionally to the 12 % CP diet, indicating that undersupply in NEAA could affect pig performance. Some studies found a reduction in N retained (g/d) with 4 to 6 %-points dietary CP reductions in 36 and 41 kg growing pigs when the diets were deficient in one or more EAA (Deng *et al.*, 2007; Figueroa *et al.*, 2002). Otto *et al.* (2003) observed improved N retention (% of intake) but N retained (g/d) was reduced when dietary CP was reduced from 14.6 to 11 or 9.9 % while total EAA:NEAA ratio of 45 % was kept. This may be partly due to the reduced level of Lys and some NEAA (Gly, Pro) in the low CP diets coupled with formulating diets on total AA basis. Regardless, all the N balance data consistently found significant reduction in total N excretion accompanied by improved in N retention (% of diet). Based on the overall 26 N-balance datasets, 1 %-point dietary CP reduction results on average 9 % reduction in N excretion in pigs.

POTENTIAL REASONS FOR THE INCONSISTENCIES OF RESULTS WITH FEEDING PIGS WITH LOW PROTEIN DIETS

A majority of the studies indicate that reducing the dietary CP at or even more than 4 %-points in diets for all pig categories is possible without detrimental effect on the performance or N retention when the diets are properly balanced for all EAA on SID basis and energy preferably on NE basis. Nevertheless, the inconsistencies of results with feeding pig low CP-AA diets in pigs exist and the affecting factors can be listed as follow:

a) Due to the fact that diets were balanced only for the first 4 limiting AA (Lys, Thr, Met and Trp) which often led to deficiency in the next limiting AA, typically Val and Ile.

b) When the dietary CP level was reduced at or greater than 6 %-points even though diets was balanced for most EAA, the contents of NEAA (particularly Gly, Glu) or N became too low (deficient) for synthesis of NEAA and other physiological functions (formation of mucosa protein and digestive enzymes affecting nutrient utilization) which was also clearly indicated by the SID Lys:CP ratio of greater than 7.0 % in the low CP-AA diets.

c) Formulating low CP-AA diets on similar “DE” or “ME” basis led to a greater backfat thickness in finishing pigs which was mainly due to more efficient energy utilization of pigs fed low CP diet coupled with the excess energy being stored as carcass fat.

TABLE 4 Contents of CP, Lys and Lys:CP ratios in pig diets (analyzed in AMINOLab® from 2010 to 2015)

	DIETS							
	PRE-STARTER		STARTER		GROWER		FINISHER	
Dietary content	EU[1] n=138a	Non-EU[2] n=646b	HP n=217	LP n=721	HP n=538	LP n=1560	HP n=94	LP n=362
CP								
mean (%)	20.13	22.02	18.83	19.77	16.43	17.52	15.04	15.50
CV (%)	9.9	9.4	7.3	6.4	8.6	9.0	20.3	15.6
Minimum (%)	12.80	14.53	14.73	16.16	12.67	12.42	9.78	8.96
Maximum (%)	25.31	33.29	22.55	25.46	20.69	22.86	20.62	21.78
Lys								
mean (%)	1.46	1.46	1.27	1.27	1.02	1.03	0.73	0.74
CV (%)	8.0	5.9	3.2	3.2	9.7	9.3	14.8	11.9
Minimum (%)	1.35	1.35	1.20	1.20	0.85	0.85	0.44	0.31
Maximum (%)	1.86	1.80	1.35	1.35	1.19	1.19	0.84	0.84
Lys:CP								
mean (%)	7.32	6.68	6.78	6.44	6.23	5.89	4.94	4.88
CV (%)	10.7	8.2	7.6	6.1	8.7	7.8	12.8	16.5
Minimum (%)	5.6	5.10	5.6	5.22	4.27	4.21	3.87	1.97
Maximum (%)	11.9	9.81	8.6	8.09	7.73	9.02	6.48	7.24

[1] EU = countries belong to the European Union.
[2] Non-EU = countries which do not belong to the European Union.

d) A majority of the studies which reported reduced performance by feeding low CP-AA diets were formulated diets on the basis of total AA content which is known to be inaccurate for balancing AA requirements or sometimes diets were not properly mixed which led to one or more EAA became limiting which were revealed by the analyzed AA results.

e) A reduction in dietary electrolyte balance (calculated as Na + K – Cl) in low CP diets, by replacing a part of soybean meal (contains 2 to 2.2 % K) with AA, may influence AA metabolism of the animals even though electrolyte balance seemed not affecting pig performance.

f) Plasma AA analysis revealed that the reduced performance and lower organ weights of starter pigs by feeding low CP-AA diet sometimes may be attributed to the fact that the plasma level of most NEAA and Arg were greatly lower (reduced AA availability for growth).

g) More rapid absorption of free AA (e.g. Lys) may cause a temporary AA imbalance at the sites of protein synthesis and consequently affect performance when fed one meal per day. However, N or energy utilization is not affected when pigs are fed at least twice per day (Le Bellego *et al.*, 2001) as it was the case for the studies covered in this review.

HOW DO THE LEVELS OF CP AND LYSINE LOOK LIKE IN COMMERCIAL PIG DIETS?

The contents of CP and Lys, and Lys:CP ratios of a large number of commercial pig diets from different countries that were analyzed in Evonik's AMINOLab® from 2010 to 2015 are summarized in Table 4. The diets for pre-starter (<10 kg BW), starter (10 to 25 kg), grower (25 to 75 kg BW) and finisher pigs (75 to 125 kg) are grouped based on Lys contents in the diets. It should be mentioned that the mean Lys contents were similar but the mean CP contents were lower (1 %-point on average across different phases) resulting a slightly greater Lys:CP ratio in pig diets produced in the European Union (EU) compared with diets coming from the Non-EU countries. Setting a maximum dietary Lys:CP ratio is a useful tool to minimize the risk of NEAA become limiting. Based on this review the maximum

TABLE 5 Effect of dietary supplemental amino acids on composition and cost of pre-starter and starter pig diets

	PRE-STARTER DIETS			STARTER DIETS		
	Lys, Met	Lys, Thr, Met, Trp	Lys, Thr, Met, Trp, Val	Lys, Met	Lys, Thr, Met, Trp	Lys, Thr, Met, Trp, Val
INGREDIENTS, %						
Wheat	5.00	15.00	21.58	15.00	15.00	15.00
Barley	15.00	15.00	5.00	15.00	15.00	15.00
Corn	15.28	15.00	20.00	21.00	28.78	29.53
Soybean meal (48 %)	45.72	36.45	35.52	40.71	33.00	33.00
Whey powder	10.00	10.00	10.00	–	–	–
Corn gluten meal	–	–	–	–	0.76	–
Soybean oil	6.14	5.08	4.39	–	–	–
Tallow	–	–	–	5.31	3.99	3.95
Others[a]	2.64	2.75	2.71	2.79	2.73	2.73
L-Lys·HCl	0.054	0.324	0.361	0.154	0.384	0.390
L-Thr	–	0.111	0.125	0.034	0.126	0.139
DL-Met	0.170	0.236	0.240	–	0.195	0.210
L-Trp	–	0.051	0.055	–	0.037	0.039
L-Val	–	–	0.017	–	–	0.016
NUTRIENT CONTENTS[b]						
NE (MJ/kg)	10.50	10.50	10.50	10.30	10.30	10.30
CP (%)	26.59	23.78	23.46	24.80	22.54	22.17
Total Lys (%)	1.57	1.55	1.54	1.47	1.45	1.45
SID Lys (%)	1.39	1.39	1.39	1.30	1.30	1.30
SID Met+Cys	0.84	0.84	0.84	0.78	0.78	0.78
SID Thr (%)	0.89	0.88	0.88	0.82	0.82	0.82
SID Trp (%)	0.30	0.31	0.31	0.27	0.27	0.27
SID Val (%)	1.09	0.95	0.95	1.00	0.89	0.89
SID Ile (%)	1.02	0.88	0.87	0.92	0.81	0.80
FEED COST (EUR/MT)[c]	**405.00**	**404.02**	**403.51**	**320.93**	**316.42**	**315.75**
Saving (EUR/MT)		0.98	1.49		4.51	5.18

[a] Includes di-Ca-phosphate, limestone, salt and mineral-vitamin premix.
[b] The SID AA and NE were balanced according to AMINOPig®
[c] Based in ingredients prices (Feedinfo; April 2015) and L-Val price of 10 EUR/kg

dietary total and SID Lys:CP ratios seems to be 7.4 % and 6.9 %, respectively. This optimal Lys:CP ratio is taken over from the Lys:CP ratio in whole body of pigs from birth to 145 kg BW which averaged 7.3 % (Mahan and Shields, 1998). The mean Lys:CP ratio was the highest at 7.3 % in the EU pre-starter diets but still within the optimal level. However, roughly 27 % and 4 % of the pre-starter diets produced in the EU and non-EU countries exceeded the maximum Lys:CP ratio of 7.4 %. This highlights a need to consider NEAA when formulating low CP-AA diets for pre-starter pigs.

LOW PROTEIN, AMINO ACIDS-FORTIFIED DIETS ARE COST EFFECTIVE

It is essential to be profitable if the animal producers are to remain in the business. The effect of dietary supplemental amino acids on the composition and cost of pre-starter and starter pig diets are shown in Table 5. Obviously, the impact of using crystalline AA on feed cost will depend on the prices of ingredients and AA used in the feed formulations. For the current simulations, the average raw materials prices of April 2015 with L-Val at 10 EUR/kg were used. As shown in Table 5, increasing use of crystalline AA up to 5 AA including L-Val in low CP diets for pre-starter and starter pigs can be cost effective.

TABLE 6 Impact of low CP, AA diets on N and slurry excretion of 25 to 115 kg pigs

	HIGH CP	MEDIUM CP	LOW CP	LOW CP
Added AA:	**–**	**Lys, Thr, Met**	**Lys, Thr, Met, Trp**	**Lys, Thr, Met, Trp, Val**
Average CP (%)	**20.3**	**16.0**	**14.7**	**13.6**
Total feed intake (kg/pig)[a]	204	204	204	204
Total N intake (kg/pig)	6.43	5.04	4.71	4.30
Total N excretion (kg/pig)[b]	2.89	1.92	1.79	1.63
For "10,000 pigs" from 25 to 115 kg production scenario				
Total N excretion (MT)	28.91	19.15	17.88	16.35
Reduction in N excretion (MT)[c]		−9.76	−11.03	−12.56
Reduction in slurry (MT)[c, d]		−1,370	−1,550	−1,770

[a] Assumed average FCR of 2.50 from 25 to 115 kg grow-out phase

[b] Assumed N retained of 55 and 62 % for high and low CP diets in 50 kg pigs (Otto *et al.*, 2003)

[c] Compared with the high CP diet

[d] Based on 7.1 kg N/ton of pig slurry (Hoeksma *et al.*, 2012)

LOW PROTEIN, AMINO ACIDS-FORTIFIED DIETS PROMOTE SUSTAINABILITY

Pigs fed low CP diet consumed less water and excreted less urine and manure relative to the high CP diet (Bellego and Noblet, 2002; Leek *et al.*, 2005). Lowering dietary CP and balancing with AA has a directly reduce the excretions of N and slurry volume. The impact of stepwise reduction of dietary CP in diets for the grow-out phase from 25 to 115 kg pigs are shown in Table 6. For the scenario of producing 10,000 pigs from 25 to 115 kg, graded level of CP reductions by using 2, 3, 4 or 5 supplemental AA in the diets would excrete 9.8, 11.0 and 12.6 metric tons (MT) less N to the environment, respectively. These are equivalent to 1,370, 1,550 and 1,770 MT less pig slurry to be stored and get rid of by the farm which can have cost saving effect for some farms with limited amount of land.

CONCLUSIONS AND IMPLICATIONS

The potential to lower the dietary CP level ultimately depends on how many supplemental AA are incorporated in the diets. The overall results of this review indicate that reducing the dietary CP at about 4 %-points for all pig categories is possible without detrimental effect on the performance, carcass quality or N retention by using 5 supplemental AA (Lys, Thr, Met, Trp and Val) which are currently available and properly balancing for all EAA on SID basis and energy preferably on NE basis. However, N or some NEAA can become limiting when the dietary CP is reduced at or more than 5 %-points particularly in diets for young pigs (Kerr and Easter, 1995; Gloaguen *et al.*, 2014). One practical way to overcome such obstacle is keeping a maximum dietary SID Lys:CP ratio of 6.9 % (7.4 % total Lys:CP) which can be achieved by supplementing with some key NEAA.

It seems that Gly and Glu are rather effective in using as NEAA sources in low CP diets (Powell *et al.*, 2011; Gloaguen *et al.*, 2014). This may be due to their multiple roles other than protein synthesis as Gly is involved in the synthesis of Ser, glutathione, creatine, purine nucleotides and heme, and Glu can serve as the precursor for synthesis of Gln, Pro, Arg, ornithine and glutathione.

Lowering dietary CP and balancing with AA is very effective in reducing the excretions of N and slurry to the environment. Based on the overall 26 N-balance datasets, 1 %-point dietary CP reduction results on average 9 % reduction in N excretion in pigs. Furthermore, pigs fed low CP diet consumed less water and excreted less urine and manure relative to the high CP diet. The progress in our understanding of low CP diets allows nutritionists to formulate diets that are cost effective and more environmentally friendly without impacting pig performance.

ACRONYMS

AA	Amino acids
ADG	Average daily gain
CP	Crude protein
DE	Digestible energy
EAA	Essential amino acids
EU	European Union
FCR	Feed conversion ratio
Gln	Glutamine
Glu	Glutamate
Gly	Glycine
His	Histidine
Ile	Isoleucine
Leu	Leucine
Lys	Lysine
ME	Metabolizable energy
Met	Methionine
MT	Metric tons
N	Nitrogen
NE	Net energy
NEAA	Nonessential amino acids
Phe	Phenylalanine
Pro	Proline
SID	Standardized ileal digestible
Thr	Threonine
Trp	Tryptophan
Val	Valine

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