Ciencia e Investigación AGRARIA

Cien. Inv. Agr. 33(2): 83-90. 2006
www.rcia.puc.cl

REVISION DE LITERATURA

Evaluation of the quality of protein sources for inclusion in diets for monogastric animals

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Abstract

S.M. Hodgkinson. 2006. Evaluation of the quality of protein sources for inclusion in diets for monogastric animals. Cien. Inv. Agr. 33(2):83-90. For the precise formulation of the protein (amino acid) component of diets for monogastric animals such as the pig and chicken, it is of vital importance to have information regarding the nutritional quality of the protein sources available. Determinations of the nutritional availability of the amino acids must be carried out with ileal digesta rather than with feces. For feedstuffs that have not undergone processing (with heat and pressure, for example), true ileal amino acid digestibility coefficients are the most appropriate for use in practical diet formulation. However, for feedstuffs that have undergone processing, the use of conventional methods to determine true ileal amino acid digestibility will overestimate the availability of amino acids such as lysine. In these cases, different methods must be used to determine the availability of lysine, such as the O-methylisourea or true reactive lysine bioassay method. The objective is to present a critical evaluation of the appropriateness of the use of the different methodologies currently available to determine the availability of amino acids in protein sources, both processed and non-processed, for the monogastric animals.

Key words: Amino acids, diet formulation, lysine, processed feedstuffs, protein.

Introduction

In the formulation of diets for monogastric animals such as the pig, ingredients included to meet the protein (amino acid) needs of the animals along with those included as energy sources, constitute the main cost of the diet (Noblet and Perez, 1993; Noblet and van Milgen, 2004). For this reason, as well as the environmental consequences of over-formulating the protein component of a diet, and the lower production levels in animals that are associated with under-formulating the protein component of a diet, it is of vital importance to have information regarding the amounts of amino acids in the protein sources that are available to the animal for metabolism.

Whereas the amino acid content of protein sources can be determined via chemical analysis (for a discussion of methodologies see Peace and Gilani, 2005), a proportion of these amino acids will not be completely digested and absorbed by the animal. Moreover, this proportion will vary between sources of protein and even between sources or batches of the same ingredient (Hendriks *et al*., 2002; 2004). Accurate information is required on the amounts of amino acids in protein sources that are available to the animal to allow precise formulation of the diets. In the present work, the term "availability" is used to refer to the amount of an amino acid that is absorbed in a chemical form that is suitable for utilization by the animal for body protein synthesis.

Accepting this definition, the digestibility and availability values for an amino acid will usually be numerically similar as most of the

Received 13 January 2006; Accepted 19 May 2006.
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amino acids will be absorbed in their native chemical form which can then be utilized by the animal. Therefore, the amino acid digestibility of a feedstuff will give a good indication of its availability. This is not always the case, however, with feedstuffs that have been subjected to processing or prolonged storage, which may provoke significant and nutritionally important structural changes. Therefore, the determination of amino acid digestibility and availability in processed and unprocessed feedstuffs will be discussed separately.

The objective of this revision is to present a critical evaluation of the appropriateness of the use of the different methodologies currently available to determine the availability of amino acids in protein sources, both processed and non-processed, for the monogastric animals.

Fecal versus ileal amino acid digestibility

Traditionally with simple-stomached animals, dietary amino acid digestibility has been determined based on fecal measurement. However, microbes are present in high numbers in the large intestine and these microbes metabolize the amino acids, changing the amino acid profile of the digesta (Just, 1980; McNeil, 1988). Both the level of fermentation and the susceptibility of exogenous and endogenous proteins to bacterial attack will determine the amount of both exogenous and endogenous amino acids appearing in the feces (Mason and Palmer, 1973). Also, amino acids are not absorbed, as such, to any significant extent in the large intestine (Just, 1983; Schmitz *et al*., 1991; Darragh *et al*., 1994) and the nitrogen that is absorbed from the large intestine (in the form of ammonia, amines and amides) is normally of no nutritional value to the animal, and is metabolized and excreted in the urine as urea (Zebrowska 1973; Just *et al*., 1981; McNeil, 1988). The determination of amino acid digestibility based on fecal collection is misleading, therefore, and for accuracy, amino acid digestibility needs to be quantified at the end of the small intestine; the terminal ileum (Sauer and Ozimek, 1986). For a review of the methods that can be applied to collect ileal digesta, see Hodgkinson and Moughan (2000). The difference between fecal and ileal digestibility coefficients for protein in different animal species are generally quite significant (Moughan and Donkoh, 1991). It is also important to note that the difference between ileal and fecal digestibility coefficients is not constant between amino acids (Rowan *et al*., 1994).

It has been demonstrated that ileal digestibility coefficients are accurate for describing the extent of amino acid uptake from the digestive tract of simple-stomached animals, at least for non heat-treated proteins (Just *et al*., 1985; Moughan and Smith, 1985).

Unprocessed feedstuffs

When the digestibility of a dietary protein source is calculated by subtracting the amount of amino acids leaving the terminal ileum from the amount of amino acids that were ingested by the animal, the "apparent" digestibility coefficient is obtained.

Many nitrogen-containing compounds, including amino acids, peptides and proteins, diffuse into or are secreted into the gastrointestinal tract during the digestion of food. These compounds are termed endogenous, as opposed to dietary or exogenous material (Fauconneau and Michel, 1970; Snook, 1973). Endogenous nitrogen-containing material includes secretions containing digestive enzymes produced by the salivary glands, pancreas and the mucosal lining of the stomach and intestines, and bile acids from the liver. Mucus is secreted from cells along the entire gastrointestinal tract, and epithelial cells are sloughed off from the intestinal mucosa. Serum albumin is also present in the gastrointestinal tract. Most (70-80%) of this endogenous nitrogen is reabsorbed before the digesta leave the small intestine (Souffrant, 1991), but the remaining 20-30% enters the large intestine.

When amino acid digestibility coefficients are calculated including a correction for basal endogenous amino acids, "true" digestibility coefficients are obtained. Values for basal endogenous amino acid losses, which must be expressed on a food dry matter intake basis (Butts *et al*., 1993), can be determined for

different species of animals using techniques such as peptide alimentation (enzyme hydrolyzed protein method, Moughan *et al*., 1990), ^{15}N isotope dilution (Souffrant *et al*., 1982; de Lange *et al*., 1990; Leterme *et al*., 1994) and the homoarginine method (Hagemeister and Erbersdobler, 1985; Rutherfurd and Moughan, 1990) when diets that do not contain fiber or ANF's are used. These three methods have been shown to give similar results of endogenous ileal nitrogen or lysine flows (Hodgkinson *et al*., 2003). The traditional method used to determine endogenous flows (the protein-free method), which involves sampling ileal digesta from animals that have received a diet that is devoid of protein for approximately 8 days, has been shown to underestimate endogenous ileal nitrogen and amino acid flows (Hodgkinson *et al*., 2000). For more information regarding methodologies that can be applied to determine endogenous ileal amino acid flows, see Moughan *et al*. (1998) and Hodgkinson and Moughan (2000).

True digestibility is a fundamental property of the food, and is not affected by the dietary conditions under which the food is given to the animal. The apparent digestibility measure, however, will be affected by the assay conditions and is, therefore, variable and open to error. True digestibility is a superior measure for determining the amino acids which are absorbed from the gut and gives a better representation of protein quality than apparent digestibility.

Many foods contain fiber and/or antinutritional factors (ANF's), and these may stimulate increased endogenous losses, above the basal levels (Schulze, 1994; Jansman *et al*., 1995; Mariscal-Landin *et al*., 1995; Schulze *et al*., 1995; Leterme *et al*., 1996). Taking these increased endogenous excretions into account (for example by use of the homoarginine or ^{15}N isotope dilution methods for determining endogenous amino acid loss with feedstuffs containing ANF´s and/or fiber) will result in "real" digestibility coefficients. In feedstuffs that do not contain ANF's or fiber, true and real digestibility coefficients will be numerically the same, and both will provide an accurate assessment of amino acid absorption. In feeds which do contain ANF's and fiber, however, only real digestibility coefficients will provide an accurate assessment of amino acid absorption, and true digestibility coefficients will underestimate the actual extent of dietary amino acid absorption.

To decide on the type of digestibility coefficient to be used in diet formulation (true digestibility or real digestibility), consideration needs to be given both to the availability of the nutrients in the feedstuff and also to the manner in which the animal's amino acid requirements have been determined. Normally the amino acid requirements of an animal are determined based on the factorial method or by the use of computerized growth simulation models (Moughan *et al*., 1995; Whittemore *et al*., 2001; Green and Whittemore, 2003; 2005). With all of these methods, endogenous amino acid losses from the animal are taken into consideration and have been factored into the requirement value. The endogenous amino acid loss value which is normally used in the determination of an animal's amino acid requirement is the general basal loss, which corresponds to the amount that will occur when the animal is receiving a diet that does not include ANF´s or fiber. Thus, this value will not include the increased endogenous amino acid loss associated with specific dietary components such as fiber and ANF's (Darragh *et al*., 1995; Boisen and Moughan, 1996).

For feedstuffs that do not contain either ANF's or fiber, the basal endogenous amino acid loss value used to calculate true digestibility will be equal in magnitude to that used in calculating the animal's amino acid requirements. Therefore, the endogenous amino acid loss value corresponding to that when the feedstuff is fed to the animal will be balanced by the equivalent value used in the estimation of the animal's amino acid requirement. True digestibility coefficients will, in this case, represent the availability of the amino acids to the animal, and are the coefficients of choice.

However, the feeding of feedstuffs which do contain ANF's or fiber to an animal will result in an increased endogenous amino acid loss from the animal, as discussed above. This increased loss will raise the animal's amino acid

requirements above that accounted for by the basal endogenous amino acid loss value that is normally used in the estimation of the animal's amino acid requirement. In this case, the use of real digestibility coefficients will result in an overestimation of the value of the feedstuff relative to the animal's amino acid requirement. Effectively, the extra endogenous amino acid loss associated with ingestion of the feedstuff is costed against the animal. Formulating the diet in this manner will lead to a decrease in efficiency of utilization and, therefore, a decrease in production. Real digestibility coefficients are only accurate for practical diet formulation if the extra endogenous amino acid loss associated with the ingestion of the feed is also used in the estimation of the animal's amino acid requirement. This is not usually the case.

If true digestibility coefficients are used in dietary formulation with feedstuffs containing ANF´s or fiber, then the additional endogenous amino acid loss associated with consumption of the feedstuff is effectively costed against the feedstuff itself. There will be an undervaluing of dietary amino acid absorption for this feedstuff. Therefore, the increased requirement for amino acids by the animal due to the additional endogenous amino acid losses, which have not been accounted for in the estimation of the amino acid requirements, will be offset by the underestimation of digestibility due to using true digestibility coefficients. This is appropriate as the additional endogenous amino acid loss is associated with the feedstuff, not the animal.

In conclusion, therefore, when animals are fed diets containing unprocessed feedstuffs, true ileal digestibility coefficients should be used in practical diet formulation. True digestibility coefficients are independent of the assay conditions used in their determination and are consistent with the way in which the amino acid requirements of the animal are generally given.

Processed feedstuffs

Many feedstuffs that are used in practical dietary formulation are processed (exposing them to heat, pressure and other materials such as alkalis) or may be stored for long periods of time. In some feedstuffs (for example soybean meal), this processing is necessary to inactivate ANF´s that are present (Gatel, 1994; Anderson and Wolf, 1995). During this processing, chemical reactions often occur between protein-bound amino acids and reducing compounds present in the feed matrix, which may render some of the amino acids nutritionally unavailable to the animal. This is particularly so for lysine. The ε-amino group of lysine can react with other compounds present in the feedstuff during processing and storage to form compounds such as deoxyketosyllysine (also called the Amadori compound), which is formed during the early-Maillard reaction. While deoxyketosyllysine is partially absorbed from the gut, it has no nutritional value to the animal (Hurrell and Carpenter, 1981). Moreover, a proportion of the reacted lysine derivatives are acid labile, and can revert back to lysine during the acid hydrolysis step of conventional amino acid analysis. This does not, however, occur in the animal's digestive tract. Consequently, the lysine concentrations of the feed and ileal digesta, determined by conventional amino acid analysis, will be overestimated and the conventional true ileal digestibility assay will generally overestimate lysine availability in heat treated feeds (Rutherfurd *et al*., 1997a; Moughan, 2005). Hence, a different approach is required.

The O-methylisourea method or true reactive lysine bioassay was proposed by Moughan and Rutherfurd (1996). This bioassay involves reacting both the diet and ileal digesta with O-methylisourea, which will convert reactive lysine (the lysine that has not been damaged with the early-Maillard reaction and thus should be available to the animal) to homoarginine. The test animals are fed the untreated feedstuff and samples of ileal digesta are collected. The diet and ileal digesta are reacted with O-methylisourea under controlled conditions, and the homoarginine contents are determined. The true ileal digestibility of reactive lysine is then calculated. These coefficients can be used to calculate the digestible reactive lysine or available lysine content of the feedstuff. The difference between this approach and previous

approaches to determine the amount of lysine available to the animal is that this approach places emphasis on determining the uptake of chemically available lysine molecules from the gut, rather than describing the uptake and utilization of chemically altered lysine molecules. It is the former that is required for dietary formulation, not the latter. For unprocessed feedstuffs the digestible reactive lysine content should be equivalent to the digestible lysine content determined using conventional methods, whereas for a processed feedstuff, the total lysine content (conventionally determined) is likely to be higher than the reactive lysine content due to the conversion of lysine derivatives to lysine during the acid hydrolysis stage of conventional amino acid analysis and total lysine digestibility will be lower. Overall, for the processed feedstuff, the digestible available lysine content will be overestimated using conventional procedures. It is important to note that in severely damaged protein sources, some of the structurally altered lysine derivatives may be acid-stable, and thus may not convert back to lysine during acid hydrolysis (Moughan, 2005). In this case, reactive and total lysine values should be more similar.

The bioassay has been shown to be more accurate than assays based on conventional amino acid analysis as an indicator of digestible reactive lysine (Rutherfurd *et al*., 1997a) and the biossay has been applied to a range of processed feedstuffs (Rutherfurd *et al*., 1997b). It can be seen from Table 1, that determination of true ileal digestibility using conventional amino acid analysis significantly underestimates lysine digestibility in ingredients such as soybean meal, dried maize, heated skim milk powder, cottonseed meal, an alfalfa-based mix and whole milk powder.

Conclusion

For the formulation of diets for monogastric animals, accurate information is required regarding the amounts of digestible, chemically-

Table 1. Comparison of mean true ileal lysine digestibility determined using conventional amino acid analysis (Total) or based on determined reactive lysine (Reactive) (Rutherfurd *et al*., 1997b).
Cuadro 1. *Digestibilidad promedio verdadera de lisina, determinada según el análisis convencional de aminoácidos (total) o determinada según lisina reactiva (reactive). (Rutherfurd* et al.*, 1997b).*

	Lysine digestibility[a], %			
	Total[b]	Reactive[c]	SE	Significance[d]
Blood meal	96.3	96.7	0.41	ns
Wheat meal	92.6	92.1	0.45	ns
Meat and bone meal	88.9	91.5	0.76	ns
Soybean meal	94.5	96.5	0.41	*
Dried maize	80.5	84.3	1.54	*
Heated skim milk powder	69.1	94.0	1.11	***
Cottonseed meal	62.1	71.9	1.75	**
Alfalfa-based mix	74.2	86.3	0.63	***
Whole milk powder	95.2	98.2	0.87	***

[a] For blood meal, wheat meal, soybean meal, meat and bone meal, heated skim milk powder and cottonseed meal, n = 8; for the dried maize and alfalfa-based mix, n=5.
Para harina de sangre, afrecho de trigo, harina de soya, harina de carne y hueso, leche descremada en polvo y harina de semilla de algodón, n=8; para maíz secado y alfalfa, n=5.
[b] Lysine digestibility was determined using a true ileal amino acid digestibility assay (rat) and conventional amino acid analysis was used to quantitate total lysine in the diets and digesta.
Digestibilidad de lisina, determinada utilizando un ensayo de digestibilidad verdadera de aminoácidos en nivel ileon (rata), lisina total en dietas y digesta determinada por análisis convencional de aminoácidos.
[c] Lysine digestibility was determined using a true ileal amino acid digestibility assay (rat), and the guanidination reaction was used to quantitate reactive lysine in the diets and digesta.
Digestibilidad de lisina determinada utilizando un ensayo de digestibilidad verdadera de aminoácidos en nivel ileon (rata) y la reacción de guanidinación utilizada para determinar lisina reactiva en dietas y digesta.
[d] *, **, ***, statistically significant at p<0.05, 0.01 and 0.001, respectively. ns= not significant (p>0.05).
, **, *, estadísticamente significativo a p<0,05; 0,01 y 0,001; respectivamente. ns=no significativo (p>0,05).*
[e] Rutherfurd and Moughan (2005).

available amino acids which can potentially be delivered to the animal from the feed. For feedstuffs that have not undergone processing or storage, the conventional true ileal digestibility assay, which gives standardized true ileal digestibility coefficients, is recommended to determine the availability of amino acids for the animal. For feedstuffs that have been processed or stored for long periods of time, at least in terms of lysine and possibly for other amino acids, the conventional true ileal digestibility assay will overestimate the amount of lysine which is available to the animal. Therefore, other methods such as the true ileal reactive lysine digestibility assay need to be applied to give accurate results.

Resumen

Para una adecuada formulación del componente proteico (aminoácidico) en dietas destinadas a animales monogástricos, como cerdos y aves, es necesario conocer la calidad nutricional de las fuentes de proteína. Con este propósito, es necesario estimar la disponibilidad de los aminoácidos de dichas fuentes de proteína. Esto se debe hacer utilizando la digestibilidad ileal, en vez de la digestibilidad fecal. Para alimentos sin procesar (ej. procesados con calor y presión) es más apropiado utilizar coeficientes de digestibilidad verdadera, determinados a nivel del íleon en la formulación de dietas. Sin embargo, en el caso de alimentos procesados, los métodos convencionales sobreestiman la disponibilidad de aminoácidos como la lisina. En estos casos, es necesario utilizar otros métodos para determinar la disponibilidad de lisina. Por ejemplo el método O-methylisourea o el bio-ensayo de lisina reactiva verdadera. Este trabajo tuvo por objetivo realizar una evaluación crítica de las metodologías actualmente existentes para determinar la disponibilidad de los aminoácidos en ingredientes, procesados y sin procesar, que son comúnmente incorporados en dietas de animales monogástricos.

Palabras clave: Alimentos procesados, aminoácidos, formulación de dietas, lisina, proteína.

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