Feeding intact proteins, peptides, or free amino acids to monogastric farm animals

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ABSTRACT

For terrestrial farm animals, intact protein sources like soybean meal have been the main ingredients providing the required amino acids (**AA**) to sustain life. However, in recent years, the availability of hydrolysed protein sources and free AA has led to the use of other forms of AA to feed farm animals. The advent of using these new forms is especially important to reduce the negative environmental impacts of animal production because these new forms allow reducing the dietary crude protein content and provide more digestible materials. However, the form in which dietary AA are provided can have an effect on the dynamics of nutrient availability for protein deposition and tissue growth including the efficiency of nutrient utilization. In this literature review, the use of different forms of AA in animal diets is explored, and their differences in digestion and absorption rates is focused on. These differences affect the postprandial plasma appearance of AA, which can have metabolic consequences, like greater insulin response when free AA or hydrolysates instead of intact proteins are fed, which can have a profound effect on metabolism and growth performance. Nevertheless, the use and application of the different AA forms in animal diets is important to achieve a more sustainable and efficient animal production system in the future, as they allow for a more precise diet formulation and reduced negative environmental impact. It is therefore important to differentiate the physiological and metabolic effects of different forms of AA to maximize their nutritional value in animal diets.

Key words: intact protein, free amino acid, hydrolysate, monogastric farm animal, animal feeding

INTRODUCTION

To become more sustainable, the negative environmental impact of farm animal production has to be reduced. This had led to global adjustments in animal feeding, like the reduction of the crude protein (**CP**) content in the diet and the better adjustment of dietary supplies to the nutritional requirements of the animal for maintenance and other physiological needs (e.g., growth, reproduction). Soybean meal is the most widely used protein source in diets of monogastric farm animals such as pigs and poultry, due to its favourable amino acid (**AA**) profile and high digestibility (Cromwell 2000). However, its use around the globe has been questioned because of the carbon and nitrogen footprint related to its production, which is mainly concentrated in the United States and Brazil. These aspects have contributed to a reduced use of soybean meal in non-producing countries and the need to use alternative, locally-sourced ingredients (Florou-Paneri et al. 2014; Garcia-Launay et al. 2014). Also, a considerable part of the protein supply of monogastric farm animals originates from cereals (e.g., corn, wheat, barley). However, the AA composition of cereals and other plant protein sources such as rapeseed meal is not as good as the AA composition of soybean meal to fulfil the AA requirement. This, in combination with the reduction of CP, has led to the use of free AA (e.g., Lys, Met, Thr, Trp, and Val) to ensure a proper supply of AA while making the animal diet cheaper and more convenient to formulate.

The production of animal-derived products (e.g., meat, milk, and eggs) also results in the production of by-products that are not used for human consumption (e.g., feathers, blood, and offals). These by-products have been used as protein sources in animal feed but the risk of pathogen transmission has reduced their use, especially in Europe. To reduce this risk, animal proteins can be hydrolysed into peptides, a shorter chain of AA, which also improves the digestibility of proteins that are resistant “by nature” such as feathers. Hydrolysis of protein

sources provides an opportunity to valorise these by-products and turn them into viable alternative AA sources in animal diets (Bah et al. 2016; Dieterich et al. 2014).

In monogastric farm animals, dietary AA can be supplied as intact proteins, peptides, or as free AA. Free AA and small peptides can be absorbed directly by the gut while intact proteins and larger peptides have to be hydrolysed through digestion in the gastrointestinal tract (Wu 1998; Krehbiel and Matthews 2003). Consequently, different dietary forms of AA are absorbed at different rates, which can have consequences on the postprandial metabolism of AA. This literature review explores the interests and perspectives of supplying dietary AA in free form, as peptides, or as intact protein, focussing on their use in the diets of monogastric farm animals like pigs and poultry. It may help to understand AA metabolism in a context of using alternative forms and sources of AA in farm animal diets.

DIGESTION OF DIETARY PROTEIN AND ABSORPTION OF AMINO ACIDS AND SMALL PEPTIDES

The provision of dietary AA is required for the production of animal-derived products as they are the building blocks of proteins constituting meat, milk, and eggs. Proteins play diverse functions such as serving as structural components (e.g., collagen, myosin, actin), as metabolic substrates, and as catalysts to various chemical reactions like enzymes (Wu 2013a; Cox and Nelson 1942). Although there are about 700 naturally occurring AA, the focus of this review is on the standard proteinogenic AA for animals, which are the 20 AA used for protein synthesis that are encoded for in the standard genetic code (Wu, 2013a; Ambrogelly et al. 2007). Selenocysteine, a rare AA found in some animal proteins (Clark et al. 2013), is not considered in this review. Peptides can be categorized by the number of AA residues they have, which ranges from di- and tri-peptides to longer oligopeptides with up to 20 AA residues. Those with even more AA residues (but less than 50 residues, corresponding to <10,000 Da molecular

77 weight) are simply called polypeptides, while a common protein has more than 50 AA residues
78 and has a more complex three-dimensional structure (Munro 2012; Ten Have et al. 2007).

79 Digestion is the breakdown of large macromolecules into absorbable nutrients (Kong and Singh
80 2008). Digestibility is the coefficient of disappearance of a nutrient from the gastrointestinal
81 tract after ingestion (Sauer and Ozimek 1986). For monogastric farm animals, protein digestion
82 involves the breakdown of dietary proteins and large peptides through hydrolysis in the stomach
83 and small intestine. The resulting small peptides and free AA are the forms that can then be
84 absorbed by the small intestine. Digestibility of protein in animal diets involves the
85 measurement of what is left of its constituent AA after passing the whole, or a section, of the
86 gastrointestinal tract. Since there is virtually no absorption of AA in the large intestine,
87 experimental methods for estimating "true" protein digestibility until the distal ileum, the last
88 section of the small intestine, have been put in place and the ileal digestibility for many available
89 ingredients used in monogastric animal diets has been determined. The protein and AA
90 digestibility of different intact protein sources varies (Table 1), which may be attributed to the
91 complexity of their protein structure or even their AA profile (Cox and Nelson 1942; Bhattarai
92 et al. 2017). Pre-hydrolysis of proteins increases their digestibility compared to the native
93 (intact) form (Heimburger et al. 1997).

94 Absorption of free AA and small peptides from the small intestinal lumen into the systemic
95 circulation involves nutrient transporters located in the enterocyte membrane. Some studies
96 show that the distribution of AA and peptide transporters in the gut is influenced by the form
97 by which the AA are provided (Morales et al. 2017). In pigs, the expression of genes coding for
98 AA transporters is upregulated when AA are provided in free form as opposed to their intact
99 protein counterparts (Morales et al. 2017; Zhang et al. 2013). Likewise, the expression of
100 intestinal PepT1, the main dietary peptide transporter in the small intestine, is upregulated in

pigs fed a diet with a high protein content, especially if the protein is highly digestible (Gilbert et al. 2008).

Protein sources with similar digestibility values do not necessarily have the same kinetics of digestion and absorption. The kinetics of digestion and absorption is difficult to study *in vitro* as it involves different and complex mechanisms and, *in vivo*, it requires multiple cannulation along the gut, which can be complicated and raises ethical concerns that limit its use. The appearance of AA in the systemic circulation after feeding may also be used to study the dynamics of digestion and absorption. However, the postprandial plasma concentration of AA reflects not only the combined dynamics of digestion and absorption, but also the metabolism by the splanchnic tissues and the protein turnover and AA metabolism of the whole organism (Liao et al. 2018). After feeding, the concentrations of AA in the peripheral blood steadily increase followed by a gradual decline (Fig. 1 and 2). The magnitude of this increase and decrease is more important when feeding rapidly absorbed AA like free AA and hydrolysed proteins. In rats (Fig. 1), gastric administration of intact proteins, hydrolysates, or free AA with the same AA profile results in a quicker appearance of AA in the plasma for free AA and hydrolysates as opposed to intact proteins. Thus, the plasma AA kinetics of free AA and hydrolysates results in an asymmetric bell-shaped curve while it was rather flat for intact proteins (Kodera et al. 2006), but this is not the case for all intact protein sources. In young men, the postprandial plasma Leu concentration reaches its maximum within one hour after ingestion of whey protein, with a kinetic curve looking similar to that of feeding free AA and hydrolysates (Fig. 2). However, feeding casein results in a kinetic curve that remained flat throughout the experiment (Dangin et al. 2001), like for intact soy proteins. Though feeding free AA and hydrolysates results in a faster appearance of AA in the plasma, the rate of appearance of AA after the ingestion of different intact proteins may be variable, which allows to classify intact proteins as so-called fast- and slow-proteins (Dangin et al. 2001). Fast-proteins

(e.g., whey) are proteins that are quickly digested and absorbed, and the released AA appear quickly in the blood. Conversely, slow-proteins (e.g., casein, soybean meal) are digested slower and their constituent AA appear with a more delayed increase in plasma postprandial concentrations (Koopman et al. 2009). Therefore, the postprandial AA kinetics of slow-proteins often look flat compared to fast-proteins.

CONSEQUENCES OF FEEDING INTACT PROTEINS, HYDROLYSATES, OR FREE AMINO ACIDS ON ANIMAL METABOLISM

The postprandial metabolic responses of animals after feeding intact proteins and protein hydrolysates or free AA are summarized in Fig. 3. The kinetics of digestion and absorption determine the metabolic fate of AA by non-digestive tissues (Wang et al. 2021a). More specifically, this fate is mainly due to the rate of AA appearance in the plasma. A rapid increase and high concentrations of AA in the peripheral circulation induced by feeding free AA or hydrolysates result in contrasting outcomes. It increases the rate of AA deamination (catabolism) by the liver (Davis et al. 2002; De Feo et al. 1992) while also enhancing protein synthesis of tissues like the muscle (Dangin et al. 2001). The increase in muscle protein synthesis is also induced by the insulin response after a meal by favouring the utilisation of AA for protein synthesis while decreasing protein breakdown (O'Connor et al. 2003; Paddon-Jones et al. 2004). Although insulin and AA act independently on protein synthesis (O'Connor et al. 2003), they synergistically stimulate muscle protein synthesis after meal ingestion. Feeding free AA or hydrolysates induces a higher postprandial peak of insulin compared to feeding intact proteins (Calbet and Holst 2004) with certain AA like Arg, Leu, Phe, and Gln being known to stimulate insulin secretion (van Loon et al. 2000; O'Connor et al. 2003). Particularly, Leu has been shown to increase the sensitivity of muscle to insulin and stimulates muscle protein synthesis (Anthony et al. 2002). Compared to feeding hydrolysates or free AA, the postprandial insulin secretion and plasma AA concentrations are lower for intact proteins (van Loon et al. 2000), which can lead to lower protein synthesis and retention (Koopman et al. 2009).

Feeding animals diets with free AA and peptides also increases AA catabolism and therefore results in an inefficient use of AA (Davis et al. 2002; Hou et al. 2017). Some studies found that feeding free AA instead of intact proteins resulted in an inefficient use of AA for protein retention (Batterham and Bayley 1989; Batterham and Murison 1981). With a sudden and quick appearance of AA in the plasma after ingestion of free AA, the sites of protein synthesis are assumed to be saturated, which can reduce the uptake and use of AA by tissues (Batterham and Bayley 1989). Since AA are not stored in the body, free AA remain in the plasma and are subject to catabolism (Boirie et al. 1997; Guillet et al. 2004). Conversely, the delayed plasma appearance of AA released by the digestion of intact proteins results in lower catabolism of AA and, even though it results in a lower rate of protein synthesis, may result in a better nitrogen balance after the meal (Boirie et al. 1997).

In practical conditions, farm animals are fed with a mixture of the different forms of AA (i.e., protein-bound and free AA). In pigs, the absorption of total Lys and Thr was lower when pigs were fed a low CP diet supplemented with free Lys and Thr than when these AA were provided in the same amount as intact proteins (Yen et al. 2004). This occurs despite a faster absorption of these two AA when supplied as free form as indicated by the maximum plasma concentrations of Lys and Thr in the portal vein that were observed two hours earlier than for pigs fed intact protein. A more gradual appearance of dietary AA in the systemic circulation as observed after ingestion of intact proteins may lead to a greater protein utilization and muscle protein accretion (Reidy et al. 2013; Wang et al. 2021a).

In the lumen of the gut, proteins of non-dietary origin are commonly referred to as “endogenous proteins”. Feeding intact proteins results in greater endogenous protein production than with feeding free AA (Nyachoti et al. 1997). The endogenous proteins are nitrogen-containing compounds like digestive enzymes, products of the mucosal membrane (e.g., mucins), and sloughed off epithelial cells (Awati et al. 2009). A significant part of these endogenous proteins

(e.g., gastric and pancreatic secretions and enzymes) is produced in response to digesta present in the gastro-intestinal tract (Lobley 2003). Feeding free AA instead of intact proteins, diminishes the secretion of proteolytic enzymes and could therefore affect the "apparent" protein digestion (Adeola et al. 2016; Butts et al. 1993). In addition, endogenous secretions can be partially re-absorbed and are not necessarily lost for the animal (Souffrant et al. 1986). Endogenous secretions thus have both a direct (i.e., providing the digestive enzymes) and an indirect effect (i.e., by contributing to protein secretion and absorption in the gut) on digestion and, eventually on plasma AA appearance.

The gut itself also uses dietary AA, thereby affecting the overall bioavailability of these AA. The portal-drained viscera, which include the stomach, intestines, pancreas, and spleen, are estimated to use 30 to 60% of AA like Thr, Lys, and Phe (Stoll and Burin 2006), while they only account for less than 10% of the body weight in monogastric animals. Some of these dietary AA are metabolized by the gut to produce other molecules. For example, the gut tissue has been shown convert dietary Met to Cys that is one of the three AA constituting glutathione, a major antioxidant in the gut (Li et al. 2014; Riedijk et al. 2007). Apart from the gut tissue, the microbiota found in the small intestinal lumen also use dietary AA (Apajalahti and Vienola 2016). In broilers, the AA requirement of lactic acid producing bacteria (i.e., *Lactobacillus* spp., *Streptococcus* spp., and *Enterococcus* spp.), which is the dominant microbiota population in the small intestine, is very close to the AA requirement of birds (Apajalahti and Vienola 2016; Dai et al. 2013a). This may mean that the gut microbiota can potentially compete with the animal for dietary AA, which might be more critical for essential AA (**EAA**). Proteins that are not digested in the small intestine are fermented in the large intestine, affecting the microbial ecosystem and favour the proliferation of harmful bacteria (Apajalahti and Vienola 2016). Conversely, feeding free AA or hydrolysed proteins that are absorbed very quickly in the small

intestine prevents the proliferation of harmful bacteria (Wang et al. 2016; Zhang and Piao 2021) and may contribute to maintain the absorptive and digestive capacities of the small intestine.

FEEDING FREE AMINO ACIDS AND HYDROLYZED PROTEINS TO COMPLEMENT INTACT PROTEINS IN ANIMAL DIETS

In terrestrial farm animal nutrition, dietary AA are mostly provided as intact proteins from soybean meal and cereal grains. Other protein sources include cereal by-products, legumes, oil seed meals, and animal-by products (Florou-Paneri et al. 2014) and the use of these sources differs between countries and geographic areas. During the last decades, there has been an increased use of free AA, which are often added to supplement and complement low-protein diets to ensure the proper supply of AA and allows formulating diets with a composition close to the requirements of the animal. Dietary AA are commonly classified as nutritionally essential or non-essential AA. The EAA are those that should be provided by the diet because of the inability of the animal to synthesize the carbon backbone and those that are insufficiently synthesized. Non-essential AA (**NEAA**) can be synthesized *de novo* using metabolic intermediates (D'Mello 2003). However, there is a difference between the biochemical and the biological capacity to synthesize NEAA. In young pigs, reducing the dietary supply of NEAA has been shown to limit protein synthesis and growth (Deng et al. 2009). Thus, even though animals may have the capacity to synthesize NEAA, they may have a dietary requirement for these AA (Wu et al. 2013) at certain production stages. For example, during pregnancy, lactation, and weaning Arg, Glu, Gln, Gly, and Pro may become limiting for pigs and require a dietary supply (Wu et al. 2014). Therefore, it is difficult to define the "essentiality" of AA because all AA are conditionally essential (Table 2).

Ideal protein is a concept used in animal nutrition to express the AA requirements of the animal. It refers to the balance of dietary AA that is needed to exactly cover the productive potential of farm animals. It concerns all the EAA and some conditionally EAA. The capacity to synthesize

the other NEAA is assumed to be sufficient to cover the requirements (Baker 2000). The AA profile of ideal protein is expressed as a ratio relative to of Lys, which is the first limiting AA in typical diets. However, it ignores possible interactions among AA (e.g., among the branched-chain AA; Kim et al. 2001; van Milgen and Dourmad, 2015). Also, the ideal protein requirement depends on the physiological state of the animal (e.g., weaned piglets, growing pigs, gestating and lactating sows, laying hens) and on the capacity to synthesize certain AA (e.g., Pro and Gly in poultry), and therefore, different ideal protein ratios have been proposed. The concept of ideal protein is simple and has been widely used in formulating diets for monogastric animals.

Use of free amino acids in farm animal nutrition

The concept of ideal protein largely contributed to the use of free AA in animal diets. Furthermore, it is believed that the CP of the diet can be reduced without affecting performance as long as the supply of EAA and NEAA is ensured (Gloaguen et al. 2014; Heo et al. 2009). Thus, the provision of synthetic EAA to complement intact proteins in the diet allows that farm animals can perform according to their genetic potential, as dictated by the ideal protein concept. The highest efficiency of nitrogen utilization is achieved when the supply of all AA exactly matches the requirement. This means that more nitrogen will be used for protein retention and production of animal derived-products and less nitrogen is excreted (Garcia-Launay et al. 2014). Providing protein sources that are not easily digested results in a lower efficiency of nitrogen utilization. Likewise, provision of AA in excess lowers nitrogen efficiency.

Free AA in pure, crystalline form like L-lysine, DL-methionine, L-threonine, L-tryptophan, and L-valine are commonly added to commercial animal diets. The L-enantiomers are produced by fermentation and subsequent purification, whereas DL-methionine is produced through chemical synthesis. Other commercially available AA include L-methionine and hydroxy

analogues of methionine. The reduction of the CP content of the diet accompanied by supplementation of free AA has been called the "protein-sparing effect of free AA" (Baker 2009; Han and Lee 2000). However, there are practical limitations to the extent of this effect. Providing a very low CP diet to broilers (4.5% lower CP than the control diet) reduced their growth performance even though the diet was supplemented by all limiting EAA according to the ideal protein concept (Wang et al. 2021b). Similarly, finishing pigs fed with diets with very low levels of CP (11 vs 14%) supplemented with EAA had lower growth performance than those fed a control diet (Tuitoek et al. 1997). Recent studies suggest that the synthesis of NEAA in pigs depends on the availability of EAA and glucose (Hou et al. 2017), both of which are primarily sourced from the diet. Also, direct supplementation of NEAA in free form may alleviate the need for their *de novo* synthesis when AA nitrogen is limiting (Gloaguen et al. 2014).

The provision of Lys, Met, and Thr in synthetic form in broiler diets resulted in higher plasma concentrations of these AA than when they were provided as intact proteins (Chrystal et al. 2020). In pigs, the Lys and Thr plasma concentrations remained higher for a longer period when these AA were provided in free form instead of intact proteins (Morales et al. 2020). High plasma concentrations after ingestion of free AA for an extended period may indicate that these AA are not used for protein synthesis, which may be a signal of inefficiency in AA utilization. This may be caused by an AA imbalance in plasma due to the rapid appearance of AA (Yen et al. 2004). Batterham and Bayley (1989) suggested that this imbalance may also result in increased AA catabolism and therefore in a lower AA efficiency of feeding diets with free AA. To summarize, the bioavailability of AA provided in free form by the diet may not be the same as that coming from intact proteins. It remains unknown if there is a ratio between AA coming from intact proteins and free AA that optimizes AA utilisation for protein retention.

There is a growing interest in the targeted utilisation of free AA for roles other than being constituents of body proteins and peptides. These AA are usually provided as free AA instead of intact proteins as their dietary amounts can only be precisely adjusted using free AA. Certain AA (e.g., Asn, Thr, Ser, and Val) have effects on the modulation of metabolic pathways (Wu 2013b, 2010), the regulation of protein synthesis and turnover, carbohydrate, and AA metabolism (Dai et al. 2013b; Wu 2010), and feed intake (Trevisi et al. 2018), while others (e.g., Arg, Gln, Glu, Gly, and Trp) are involved in signalling, immunity, and antioxidative responses (Wu et al. 2009; Wu 2010). The supplementation of Gln in the diet of young broiler chickens has been shown to improve intestinal health with longer villi length, greater intestinal IgG and IgA concentrations, and improved growth performance compared to birds fed a control diet (Chalvon-Demersay et al. 2021; Bartell and Batal 2007). Stress hormones and stress-related behaviour were also reduced in young chicks when given diets that were supplemented with Ala, Ser, and Pro (Kurauchi et al. 2009). Otherwise, AA like Trp, Gln, Leu, and Ala can support health and can be adjusted based on age and the health status of animals (Le Floc'h et al. 2018).

Hydrolysed proteins or peptides in farm animal nutrition

The hydrolysis of intact protein sources opens up the possibility of using inedible, waste, or by-products in animal diets and therefore reduce competition with the food supply for humans, while also increasing the nutrient value of common ingredients by making their protein fragment more digestible (Hou et al. 2017). These ingredients are collectively called hydrolysed proteins or hydrolysates, which essentially means that they provide AA as peptides instead of intact proteins. Hydrolysis of intact protein sources improves their nutritional value and also reduces anti-nutritional factors that may be present (Martínez-Alvarez et al. 2015; Pasupuleti and Braun 2008). For soybean meal and other highly digestible materials, the improvement may be marginal, but pre-hydrolysis of materials that are not easily digested improves their nutritional value substantially. Hydrolysis of non-edible materials like feathers (with a protein

digestibility of less than 10%) results in a five-fold increase of its protein digestibility while a more intensive hydrolysis process renders the feathers almost completely digestible (Grazziotin et al. 2006). Hydrolysis techniques by chemical (i.e., acidic or basic) or physical means (i.e., steam and pressure) are used for both animal-derived proteins (e.g., casein, whey, blood, meat, mucosa) and plant-based proteins (e.g., soybean, wheat, barley; Pasupuleti et al. 2008; Dieterich et al. 2014). Keratin-based materials (e.g., feathers, wool, and hair) can be processed through these means and can also be treated by controlled bacterial breakdown (Pasupuleti and Braun 2008). Other protein sources can be processed through microbial fermentation techniques that not only improves digestibility but also modifies the AA profile of the material (Cervantes-Pahm and Stein 2010; Jones et al. 2010). Extensive hydrolysis results in a greater proportion of small peptides and free AA compared to those that were only partially or briefly hydrolysed (Bouhamed and Kechaou 2017), but it may also result in the degradation of the AA (Bellagamba et al. 2015; Papadopoulos et al. 1986). Certain hydrolysis conditions induce crosslinking of AA or racemization with a negative impact on digestibility (Bouhamed and Kechaou 2017; Friedman 1999).

Like certain free AA, peptide-rich ingredients can have properties other than being a highly digestible AA source. Peptides with less than 20 AA residues and abundant in Arg, Lys, and Pro residues induce beneficial effects like immune modulation and antimicrobial activities (López-Barrios et al. 2014). Hydrolysed protein products have been tested for their immunomodulation and anti-oxidant properties (Nørgaard et al. 2012; Hou et al. 2017) and as alternatives to antimicrobial to support health and growth performance of animals (Hou et al. 2017). However, the benefits on growth performance of hydrolysed proteins in animal feeds is inconsistent and their functional properties are not fully understood (Nørgaard et al. 2012; Martínez-Alvarez et al. 2015). These functional properties have been attributed to bioactive peptides such as opioid peptides (4 to 8 AA residues) that alter feeding behaviour by binding

to certain receptors in the brain (Hou et al. 2017). In addition, some bioactive peptides exert antimicrobial effects by damaging the membrane of harmful bacteria or directly affecting their metabolism (Hou et al. 2017; López-Barrios et al. 2014). Protein hydrolysates are usually fed to young animals with high AA requirements and with a less mature immune and digestive system like weaned piglets and chicks (Min et al. 2004; Hou et al. 2017). Young broiler chickens fed a diet supplemented with hydrolysed pig mucosa had greater performance compared to those fed the control diet (Frikha et al. 2014), but no effect was observed in older chickens. This might be because younger animals are more susceptible to the functional properties of hydrolysates as they are still developing their immune and digestive systems. Poudel et al. (2020) reported changes in the faecal microbiota of young pigs fed a peptide-based feed additive and hypothesised that such changes may favour the maturity of the digestive tract.

Chemical and physical hydrolysis techniques involve processes that indiscriminately cut the protein into smaller fragments. As a result, the resulting product may have different peptides in terms of size and AA composition, which may explain the inconsistencies on the observed effects on the animal. Thus, animal performance may vary substantially and enhanced performance cannot be pinpointed to a particular peptide in the hydrolysate. There is a general understanding regarding the positive effects of hydrolysis of protein sources, but further characterisation of the resulting peptides is still needed. This information is important because it may provide "additional value" to these ingredients over and above to the value of a highly digestible source of AA.

CONCLUSION

Although farm animal diets contain intact proteins, there has been an increasing trend to substitute and complete the diet with free AA or hydrolysates. Though feeding these new forms has advantages, there is still much to be learned. A better characterization of the peptides from currently available sources is needed to identify the nutritional and functional properties of

peptides. Future research should identify and quantify how the dietary form of AA affects metabolism. For example, it is unknown if the animal can metabolically differentiate between free and protein-bound dietary AA. Most of the recent studies regarding dietary form of AA deal with their short-term effects, but there is limited information on the long-term effects on metabolism and physiology. For nutritionists, the challenge will be to determine the ideal proportion of AA provided as intact proteins or in free form for optimizing animal performance and health in a sustainable way.

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TABLES AND FIGURES

Table 1. Protein and amino acid digestibility (%) of different protein sources fed to monogastric farm animals.[a,b]

Protein Source	CP	LYS	MET	TAA	Reference/s
Soybean Meal	70.0	79.9	80.1	74.4	Cervantes-Pahm and Stein. (2010)[d]
Fermented Soy	70.1	72.7	83.7	76.5	Cervantes-Pahm and Stein. (2010)[d]
Wheat Gluten	88.6	77.6	83.2	82.7	Chae et al. (1999)[c]
Fish Meal	70.8	82.9	86.0	75.2	Cervantes-Pahm and Stein. (2010)[d]
Whey Protein		93.3	89.9		Gottlob et al. (2006)[c]
Meat & Bone Meal	67.8	56.5	65.6	67.8	Wang et al. (2018)[d]
Spray-Dried Plasma	81.8	81.2	82.4	82.3	Jeong et al. (2016)[c]
Skim Milk	81.7	83.8	85.8	83.6	Chae et al. (1999)[c]
Casein	81.3	92.6	95.1	84.7	Cervantes-Pahm and Stein. (2010)[d]
Feather meal	57.8	62.0	66.5		Kerr et al. (2019) and Grazziotin et al. (2006)[d]
Faba beans	89.0	81.2	55.0		van der Peet-Schwering et al. (2006)[d]
Peas	91.9	90.9	66.8		van der Peet-Schwering et al. (2006)[d]
Canola meal	72.3	66.4	70.3		van der Peet-Schwering et al. (2006)[d]

[a] Summary of the ileal digestibility of some dietary protein sources. Free amino acids are not included in the table as they are considered to be completely digestible
[b] Values are presented as percentage for crude protein (CP), lysine (LYS), methionine (MET), and total amino acids (TAA).
[c] Apparent ileal digestible values – measured by the proportion of ingested AA left in the distal ileum
[d] Standardized ileal digestible values - Apparent ileal digestibility corrected for endogenous losses

Table 2. Nutritional classification of proteinogenic amino acids in the diets of monogastric farm animals.[a]

Mammals[b]			Poultry[c]		
EAA	CEAA	NEAA	EAA	CEAA	NEAA
Cys	Gln	Ala	Arg	Gln	Ala
His	Glu	Asn	Cys	Glu	Asn
Ile	Gly	Asp	Gly	Cys[e]	Asp
Leu	Pro	Ser	His	Tyr[f]	Ser
Lys	Arg[d]		Ile		
Met	Cys[e]		Leu		
Phe	Tyr[f]		Lys		
Thr			Met		
Trp			Phe		
Tyr			Pro		
Val			Thr		
			Trp		
			Tyr		
			Val		

[a] Table derived from Wu *et al*. (2014). Amino acids are listed in their three-letter IUPAC abbreviation. EAA – Essential amino acids, CEAA – Conditionally essential amino acids; NEAA – Non-essential amino acids
[b] Includes pigs and ruminants, also applicable for rodents
[c] Includes chickens, ducks, quails, and geese
[d] Arginine is classified as a conditionally essential amino acid for pig by NRC (2012)
[e] Can be synthesized in the liver by conversion of methionine and serine, but methionine needs to be in sufficient amount
[f] Can be synthesized in the liver by conversion of phenylalanine as long as it is in sufficient amounts

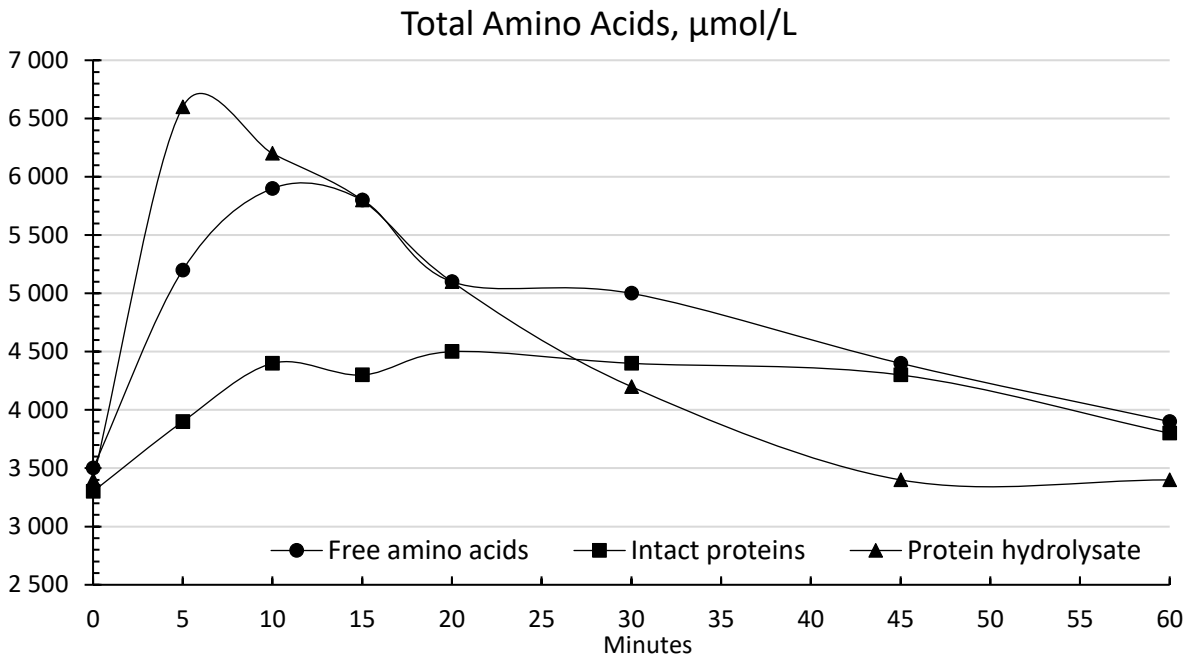

Fig. 1 Plasma concentrations of total proteinogenic amino acids (µmol/L) in the portal vein of rats after gastric infusion of intact protein (■), hydrolysate (▲), or free amino acids (●) with the same amino acid profile (Kodera et al. 2006)

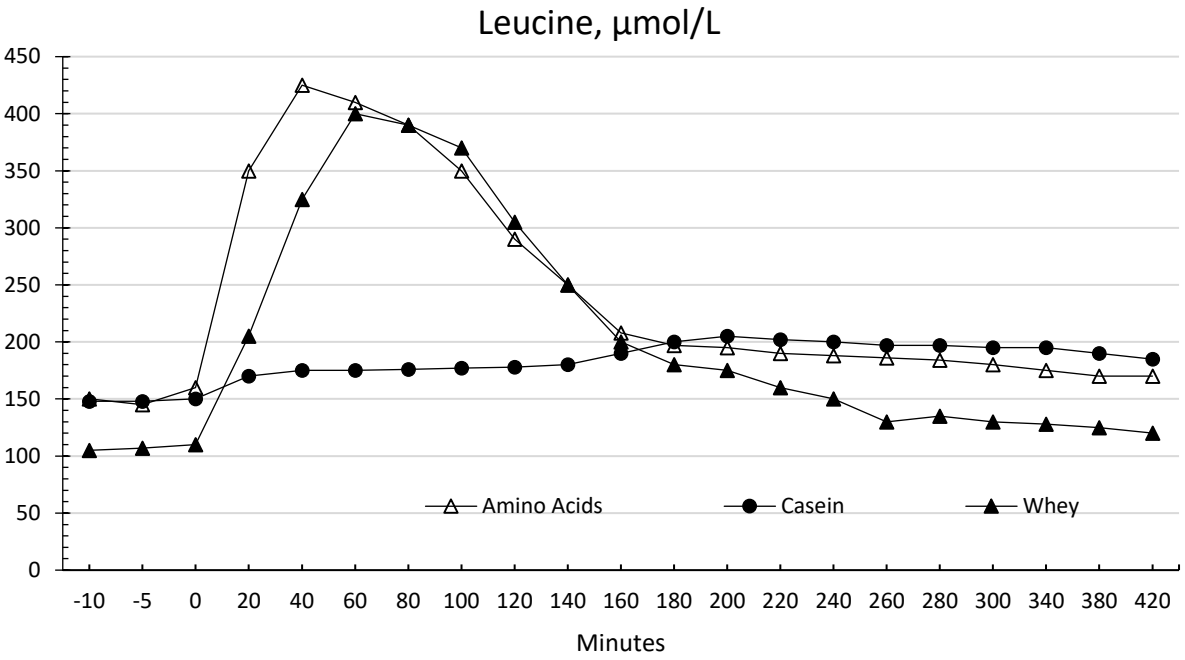

Fig. 2 Postprandial leucine plasma concentration (µmol/L) of healthy young men given free amino acids (△), whey protein (▲), and casein (●) protein sources with identical amino acid profile (Dangin et al. 2001)

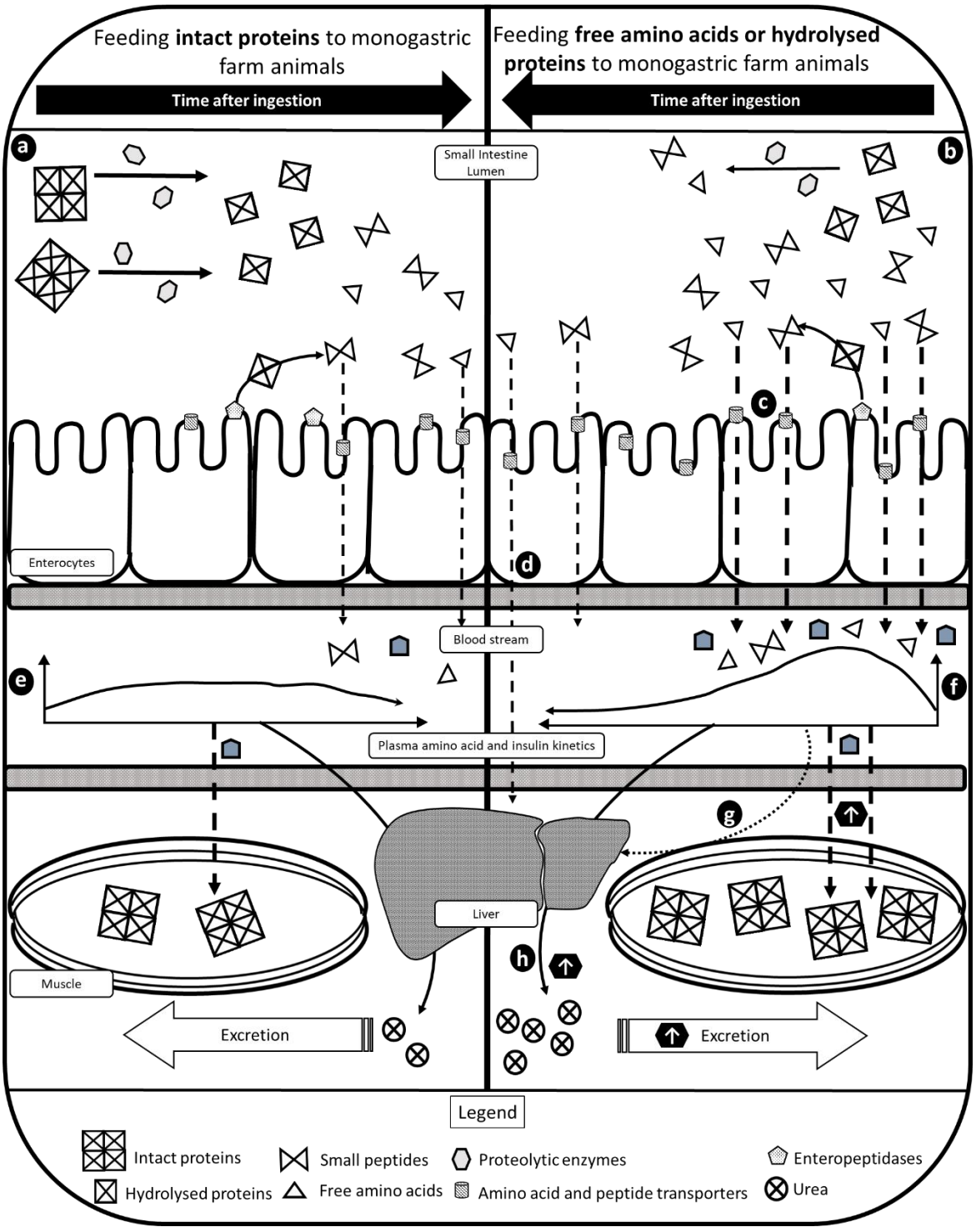

Fig. 3 Model of the different metabolic and physiological effects of feeding monogastric farm animals with different forms of dietary amino acid that is given as intact protein or as hydrolysed protein or free amino acids. In the intestinal level, because intact proteins still needs to be digested, more proteolytic enzymes are released after its ingestion contributing to more endogenous protein losses (a) while feeding hydrolysates or free amino acids lessens the need for these enzymes as hydrolysates are more easily digested and free amino acids are immediately available for absorption (b). Furthermore, due to the greater availability of hydrolysates and free amino acids, there are more amino acid and peptide transporters found

in the proximal part of the small intestine (c) although with this increased substrate availability, amino acids may be directed to the liver for deamination/catabolism (d). Since protein digestion takes time, the appearance of its products in the plasma is more constant resulting in a characteristically flat kinetics of amino acids after feeding intact proteins (e). Feeding hydrolysates and free amino acids induces a quicker appearance of plasma amino acids and an asymmetric distribution of postprandial plasma amino acids kinetics that is skewed towards the beginning (f). Plasma amino acids induces the release of insulin that increases the uptake of amino acids by the muscle and increase protein synthesis. However, in this postprandial state, due to the rapid appearance of amino acids in the plasma after feeding hydrolysates or free amino acids, there is a period of temporary imbalance of amino acid uptake of sites of protein synthesis, like the muscle, that causes more amino acids to be catabolized compared to feeding intact proteins (g). As a consequence to this increased rate of amino acid catabolism, more urea is produced by the liver which can also be interpreted as a lower efficiency of use of dietary nitrogen (h)