



**HAL**  
open science

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

F. A. Eugenio, Jaap J. van Milgen, J. Duperray, R. Sergheraert, N. Le Floc'h, Nathalie Le Floc'H

► **To cite this version:**

F. A. Eugenio, Jaap J. van Milgen, J. Duperray, R. Sergheraert, N. Le Floc'h, et al.. Feeding intact proteins, peptides, or free amino acids to monogastric farm animals. *Amino Acids*, 2022, 54, pp.157-168. 10.1007/s00726-021-03118-0 . hal-03564426

**HAL Id: hal-03564426**

**<https://hal.inrae.fr/hal-03564426>**

Submitted on 11 Feb 2022

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

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

2

3    FA Eugenio<sup>1,2</sup>, J van Milgen<sup>1</sup>, J Duperray<sup>2</sup>, R Sergheraert<sup>2</sup>, N Le Floc'h<sup>1,3</sup>

4    <sup>1</sup>PEGASE, INRAE, Institut Agro, 35590, Saint Gilles, France

5    <sup>2</sup>BCF Life Sciences, Boisel, 56140 Pleucadeuc, France

6    <sup>3</sup>Corresponding author: [nathalie.lefloch@inrae.fr](mailto:nathalie.lefloch@inrae.fr)

## ABSTRACT

7  
8 For terrestrial farm animals, intact protein sources like soybean meal have been the main  
9 ingredients providing the required amino acids (AA) to sustain life. However, in recent years,  
10 the availability of hydrolysed protein sources and free AA has led to the use of other forms of  
11 AA to feed farm animals. The advent of using these new forms is especially important to reduce  
12 the negative environmental impacts of animal production because these new forms allow  
13 reducing the dietary crude protein content and provide more digestible materials. However, the  
14 form in which dietary AA are provided can have an effect on the dynamics of nutrient  
15 availability for protein deposition and tissue growth including the efficiency of nutrient  
16 utilization. In this literature review, the use of different forms of AA in animal diets is explored,  
17 and their differences in digestion and absorption rates is focused on. These differences affect  
18 the postprandial plasma appearance of AA, which can have metabolic consequences, like  
19 greater insulin response when free AA or hydrolysates instead of intact proteins are fed, which  
20 can have a profound effect on metabolism and growth performance. Nevertheless, the use and  
21 application of the different AA forms in animal diets is important to achieve a more sustainable  
22 and efficient animal production system in the future, as they allow for a more precise diet  
23 formulation and reduced negative environmental impact. It is therefore important to  
24 differentiate the physiological and metabolic effects of different forms of AA to maximize their  
25 nutritional value in animal diets.

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

28

## INTRODUCTION

29

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

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

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

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

#### 64 **DIGESTION OF DIETARY PROTEIN AND ABSORPTION OF AMINO ACIDS AND** 65 **SMALL PEPTIDES**

66 The provision of dietary AA is required for the production of animal-derived products as they  
67 are the building blocks of proteins constituting meat, milk, and eggs. Proteins play diverse  
68 functions such as serving as structural components (e.g., collagen, myosin, actin), as metabolic  
69 substrates, and as catalysts to various chemical reactions like enzymes (Wu 2013a; Cox and  
70 Nelson 1942). Although there are about 700 naturally occurring AA, the focus of this review is  
71 on the standard proteinogenic AA for animals, which are the 20 AA used for protein synthesis  
72 that are encoded for in the standard genetic code (Wu, 2013a; Ambrogelly et al. 2007).  
73 Selenocysteine, a rare AA found in some animal proteins (Clark et al. 2013), is not considered  
74 in this review. Peptides can be categorized by the number of AA residues they have, which  
75 ranges from di- and tri-peptides to longer oligopeptides with up to 20 AA residues. Those with  
76 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

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

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

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

### 131 **CONSEQUENCES OF FEEDING INTACT PROTEINS, HYDROLYSATES, OR** 132 **FREE AMINO ACIDS ON ANIMAL METABOLISM**

133 The postprandial metabolic responses of animals after feeding intact proteins and protein  
134 hydrolysates or free AA are summarized in Fig. 3. The kinetics of digestion and absorption  
135 determine the metabolic fate of AA by non-digestive tissues (Wang et al. 2021a). More  
136 specifically, this fate is mainly due to the rate of AA appearance in the plasma. A rapid increase  
137 and high concentrations of AA in the peripheral circulation induced by feeding free AA or  
138 hydrolysates result in contrasting outcomes. It increases the rate of AA deamination  
139 (catabolism) by the liver (Davis et al. 2002; De Feo et al. 1992) while also enhancing protein  
140 synthesis of tissues like the muscle (Dangin et al. 2001). The increase in muscle protein  
141 synthesis is also induced by the insulin response after a meal by favouring the utilisation of AA  
142 for protein synthesis while decreasing protein breakdown (O'Connor et al. 2003; Paddon-Jones  
143 et al. 2004). Although insulin and AA act independently on protein synthesis (O'Connor et al.  
144 2003), they synergistically stimulate muscle protein synthesis after meal ingestion. Feeding free  
145 AA or hydrolysates induces a higher postprandial peak of insulin compared to feeding intact  
146 proteins (Calbet and Holst 2004) with certain AA like Arg, Leu, Phe, and Gln being known to  
147 stimulate insulin secretion (van Loon et al. 2000; O'Connor et al. 2003). Particularly, Leu has  
148 been shown to increase the sensitivity of muscle to insulin and stimulates muscle protein  
149 synthesis (Anthony et al. 2002). Compared to feeding hydrolysates or free AA, the postprandial  
150 insulin secretion and plasma AA concentrations are lower for intact proteins (van Loon et al.  
151 2000), which can lead to lower protein synthesis and retention (Koopman et al. 2009).



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

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

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

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

185 The gut itself also uses dietary AA, thereby affecting the overall bioavailability of these AA.  
186 The portal-drained viscera, which include the stomach, intestines, pancreas, and spleen, are  
187 estimated to use 30 to 60% of AA like Thr, Lys, and Phe (Stoll and Burin 2006), while they  
188 only account for less than 10% of the body weight in monogastric animals. Some of these  
189 dietary AA are metabolized by the gut to produce other molecules. For example, the gut tissue  
190 has been shown convert dietary Met to Cys that is one of the three AA constituting glutathione,  
191 a major antioxidant in the gut (Li et al. 2014; Riedijk et al. 2007). Apart from the gut tissue, the  
192 microbiota found in the small intestinal lumen also use dietary AA (Apajalahti and Vienola  
193 2016). In broilers, the AA requirement of lactic acid producing bacteria (i.e., *Lactobacillus* spp.,  
194 *Streptococcus* spp., and *Enterococcus* spp.), which is the dominant microbiota population in  
195 the small intestine, is very close to the AA requirement of birds (Apajalahti and Vienola 2016;  
196 Dai et al. 2013a). This may mean that the gut microbiota can potentially compete with the  
197 animal for dietary AA, which might be more critical for essential AA (**EAA**). Proteins that are  
198 not digested in the small intestine are fermented in the large intestine, affecting the microbial  
199 ecosystem and favour the proliferation of harmful bacteria (Apajalahti and Vienola 2016).  
200 Conversely, feeding free AA or hydrolysed proteins that are absorbed very quickly in the small

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

### 203 **FEEDING FREE AMINO ACIDS AND HYDROLYZED PROTEINS TO** 204 **COMPLEMENT INTACT PROTEINS IN ANIMAL DIETS**

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

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

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

#### 235 *Use of free amino acids in farm animal nutrition*

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

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

251 analogues of methionine. The reduction of the CP content of the diet accompanied by  
252 supplementation of free AA has been called the “protein-sparing effect of free AA” (Baker  
253 2009; Han and Lee 2000). However, there are practical limitations to the extent of this effect.  
254 Providing a very low CP diet to broilers (4.5% lower CP than the control diet) reduced their  
255 growth performance even though the diet was supplemented by all limiting EAA according to  
256 the ideal protein concept (Wang et al. 2021b). Similarly, finishing pigs fed with diets with very  
257 low levels of CP (11 vs 14%) supplemented with EAA had lower growth performance than  
258 those fed a control diet (Tuitoek et al. 1997). Recent studies suggest that the synthesis of NEAA  
259 in pigs depends on the availability of EAA and glucose (Hou et al. 2017), both of which are  
260 primarily sourced from the diet. Also, direct supplementation of NEAA in free form may  
261 alleviate the need for their *de novo* synthesis when AA nitrogen is limiting (Gloaguen et al.  
262 2014).

263 The provision of Lys, Met, and Thr in synthetic form in broiler diets resulted in higher plasma  
264 concentrations of these AA than when they were provided as intact proteins (Chrystal et al.  
265 2020). In pigs, the Lys and Thr plasma concentrations remained higher for a longer period when  
266 these AA were provided in free form instead of intact proteins (Morales et al. 2020). High  
267 plasma concentrations after ingestion of free AA for an extended period may indicate that these  
268 AA are not used for protein synthesis, which may be a signal of inefficiency in AA utilization.  
269 This may be caused by an AA imbalance in plasma due to the rapid appearance of AA (Yen et  
270 al. 2004). Batterham and Bayley (1989) suggested that this imbalance may also result in  
271 increased AA catabolism and therefore in a lower AA efficiency of feeding diets with free AA.  
272 To summarize, the bioavailability of AA provided in free form by the diet may not be the same  
273 as that coming from intact proteins. It remains unknown if there is a ratio between AA coming  
274 from intact proteins and free AA that optimizes AA utilisation for protein retention.

275 There is a growing interest in the targeted utilisation of free AA for roles other than being  
276 constituents of body proteins and peptides. These AA are usually provided as free AA instead  
277 of intact proteins as their dietary amounts can only be precisely adjusted using free AA. Certain  
278 AA (e.g., Asn, Thr, Ser, and Val) have effects on the modulation of metabolic pathways (Wu  
279 2013b, 2010), the regulation of protein synthesis and turnover, carbohydrate, and AA  
280 metabolism (Dai et al. 2013b; Wu 2010), and feed intake (Trevisi et al. 2018), while others  
281 (e.g., Arg, Gln, Glu, Gly, and Trp) are involved in signalling, immunity, and antioxidative  
282 responses (Wu et al. 2009; Wu 2010). The supplementation of Gln in the diet of young broiler  
283 chickens has been shown to improve intestinal health with longer villi length, greater intestinal  
284 IgG and IgA concentrations, and improved growth performance compared to birds fed a control  
285 diet (Chalvon-Demersay et al. 2021; Bartell and Batal 2007). Stress hormones and stress-related  
286 behaviour were also reduced in young chicks when given diets that were supplemented with  
287 Ala, Ser, and Pro (Kurauchi et al. 2009). Otherwise, AA like Trp, Gln, Leu, and Ala can support  
288 health and can be adjusted based on age and the health status of animals (Le Floc'h et al. 2018).

### 289 *Hydrolysed proteins or peptides in farm animal nutrition*

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

300 digestibility of less than 10%) results in a five-fold increase of its protein digestibility while a  
301 more intensive hydrolysis process renders the feathers almost completely digestible (Grazziotin  
302 et al. 2006). Hydrolysis techniques by chemical (i.e., acidic or basic) or physical means (i.e.,  
303 steam and pressure) are used for both animal-derived proteins (e.g., casein, whey, blood, meat,  
304 mucosa) and plant-based proteins (e.g., soybean, wheat, barley; Pasupuleti et al. 2008; Dieterich  
305 et al. 2014). Keratin-based materials (e.g., feathers, wool, and hair) can be processed through  
306 these means and can also be treated by controlled bacterial breakdown (Pasupuleti and Braun  
307 2008). Other protein sources can be processed through microbial fermentation techniques that  
308 not only improves digestibility but also modifies the AA profile of the material (Cervantes-  
309 Pahn and Stein 2010; Jones et al. 2010). Extensive hydrolysis results in a greater proportion of  
310 small peptides and free AA compared to those that were only partially or briefly hydrolysed  
311 (Bouhamed and Kechaou 2017), but it may also result in the degradation of the AA (Bellagamba  
312 et al. 2015; Papadopoulos et al. 1986). Certain hydrolysis conditions induce crosslinking of AA  
313 or racemization with a negative impact on digestibility (Bouhamed and Kechaou 2017;  
314 Friedman 1999).

315 Like certain free AA, peptide-rich ingredients can have properties other than being a highly  
316 digestible AA source. Peptides with less than 20 AA residues and abundant in Arg, Lys, and  
317 Pro residues induce beneficial effects like immune modulation and antimicrobial activities  
318 (López-Barrios et al. 2014). Hydrolysed protein products have been tested for their  
319 immunomodulation and anti-oxidant properties (Nørgaard et al. 2012; Hou et al. 2017) and as  
320 alternatives to antimicrobial to support health and growth performance of animals (Hou et al.  
321 2017). However, the benefits on growth performance of hydrolysed proteins in animal feeds is  
322 inconsistent and their functional properties are not fully understood (Nørgaard et al. 2012;  
323 Martínez-Alvarez et al. 2015). These functional properties have been attributed to bioactive  
324 peptides such as opioid peptides (4 to 8 AA residues) that alter feeding behaviour by binding

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

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

## 345 **CONCLUSION**

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



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

## STATEMENTS AND DECLARATIONS

357

358 **Funding:** The review paper was supported by Institut national de recherche pour l'agriculture,  
359 l'alimentation, et l'environnement (INRAE) and BCF Life Sciences (Pleucadeuc,  
360 France) as part of the Convention Industrielle de Formation par la Recherche  
361 (CIFRE) PhD grant program of F.A. Eugenio

362 **Conflicts of interest/Competing interests:** The authors have no relevant financial or non-  
363 financial interests to disclose

364 **Availability of data and material:** Not applicable

365 **Data, material and/or code availability:** Not applicable

366 **Author's contributions:** Francis Amann Eugenio performed the literature search and draft.  
367 Nathalie Le Floc'h, Jacob van Milgen, Joël Duperray, and Renaud Sergheraert  
368 conceptualized the paper and performed critical revisions on the work.

369 **Ethics approval:** This is a review paper with no experimentations; therefore, no ethical  
370 approval is required

371 **Research involving human participants and/or animals:** Not applicable

372 **Informed consent/consent to participate:** Not applicable

- 374 Adeola O, Xue P, Cowieson A, Ajuwon K (2016) Basal endogenous losses of amino acids in  
375 protein nutrition research for swine and poultry. *Anim Feed Sci Technol* 221:274-283.  
376 <https://doi.org/10.1016/j.anifeedsci.2016.06.004>
- 377 Ambrogelly A, Palioura S, Söll D (2007) Natural expansion of the genetic code. *Nat Chem Biol*  
378 3 (1):29-35. <https://doi.org/10.1038/nchembio847>
- 379 Anthony JC, Lang CH, Crozier SJ, Anthony TG, MacLean DA, Kimball SR, Jefferson LS  
380 (2002) Contribution of insulin to the translational control of protein synthesis in skeletal  
381 muscle by leucine. *Am J Physiol Endocrinol Metab* 282 (5):E1092-E1101.  
382 <https://doi.org/10.1152/ajpendo.00208.2001>
- 383 Apajalahti J, Vienola K (2016) Interaction between chicken intestinal microbiota and protein  
384 digestion. *Anim Feed Sci Technol* 221:323–330.  
385 <https://doi.org/https://doi.org/10.1016/j.anifeedsci.2016.05.004>
- 386 Awati A, Rutherford SM, Plugge W, Reynolds GW, Marrant H, Kies AK, Moughan PJ (2009)  
387 Ussing chamber results for amino acid absorption of protein hydrolysates in porcine  
388 jejunum must be corrected for endogenous protein. *J Sci Food Agric* 89 (11):1857-1861.  
389 <https://doi.org/10.1002/jsfa.3662>
- 390 Bah CS, Carne A, McConnell MA, Mros S, Bekhit AE-DA (2016) Production of bioactive  
391 peptide hydrolysates from deer, sheep, pig and cattle red blood cell fractions using plant  
392 and fungal protease preparations. *Food Chem* 202:458-466.  
393 <https://doi.org/10.1016/j.foodchem.2016.02.020>
- 394 Baker D (2000) Recent advances in use of the ideal protein concept for swine feed formulation.  
395 *Asian-australas J Anim Sci* 13:294-301.
- 396 Baker D (2009) Advances in protein–amino acid nutrition of poultry. *Amino Acids* 37 (1):29-  
397 41. <https://doi.org/10.1007/s00726-008-0198-3>
- 398 Bartell S, Batal A (2007) The effect of supplemental glutamine on growth performance,  
399 development of the gastrointestinal tract, and humoral immune response of broilers.  
400 *Poult Sci* 86 (9):1940-1947. <https://doi.org/10.1093/ps/86.9.1940>
- 401 Batterham E, Bayley H (1989) Effect of frequency of feeding of diets containing free or protein-  
402 bound lysine on the oxidation of [14c] lysine or [14c] phenylalanine by growing pigs.  
403 *Br J Nutr* 62 (3):647-655. <https://doi.org/10.1079/BJN19890065>
- 404 Batterham E, Murison R (1981) Utilization of free lysine by growing pigs. *Br J Nutr* 46 (1):87-  
405 92. <https://doi.org/10.1079/BJN19810011>
- 406 Bellagamba F, Caprino F, Mentasti T, Vasconi M, Moretti VM (2015) The impact of processing  
407 on amino acid racemization and protein quality in processed animal proteins of poultry  
408 origin. *Ital J Anim Sci* 14 (2):3770. <https://doi.org/10.4081/ijas.2015.3770>
- 409 Bhattarai RR, Dhital S, Wu P, Chen XD, Gidley MJ (2017) Digestion of isolated legume cells  
410 in a stomach-duodenum model: Three mechanisms limit starch and protein hydrolysis.  
411 *Food Funct* 8 (7):2573-2582. <https://doi.org/10.1039/C7FO00086C>

- 412 Boirie Y, Dangin M, Gachon P, Vasson M-P, Maubois JL, Beaufrère B (1997) Slow and fast  
413 dietary proteins differently modulate postprandial protein accretion. Proceedings of the  
414 National Academy of Sciences Dec 1997 94 (26):14930-14935.  
415 <https://doi.org/10.1073/pnas.94.26.14930>
- 416 Bouhamed SBH, Kechaou N (2017) Kinetic study of sulphuric acid hydrolysis of protein  
417 feathers. *Bioprocess Biosyst Eng* 40 (5):715-721. <https://doi.org/10.1007/s00449-017-1737-7>
- 419 Butts CA, Moughan PJ, Smith WC, Carr DH (1993) Endogenous lysine and other amino acid  
420 flows at the terminal ileum of the growing pig (20 kg bodyweight): The effect of protein-  
421 free, synthetic amino acid, peptide and protein alimentation. *J Sci Food Agric* 61 (1):31-  
422 40. <https://doi.org/10.1002/jsfa.2740610106>
- 423 Calbet JA, Holst JJ (2004) Gastric emptying, gastric secretion and enterogastrone response after  
424 administration of milk proteins or their peptide hydrolysates in humans. *Eur J Nutr* 43  
425 (3):127-139. <https://doi.org/10.1007/s00394-004-0448-4>
- 426 Cervantes-Pahm S, Stein H (2010) Ileal digestibility of amino acids in conventional, fermented,  
427 and enzyme-treated soybean meal and in soy protein isolate, fish meal, and casein fed  
428 to weanling pigs. *J Anim Sci* 88 (8):2674-2683. <https://doi.org/10.2527/jas.2009-2677>
- 429 Chae B, Han IK, Kim J, Yang C, Hancock J, Kim I, Anderson D (1999) Effects of dietary  
430 protein sources on ileal digestibility and growth performance for early-weaned pigs.  
431 *Livest Prod Sci* 58 (1):45-54. [https://doi.org/10.1016/S0301-6226\(98\)00184-5](https://doi.org/10.1016/S0301-6226(98)00184-5)
- 432 Chalvon-Demersay T, Luise D, Le-Floc'h N, Tesseraud S, Lambert W, Bosi P, Trevisi P,  
433 Beaumont M, Corrent E (2021) Functional amino acids in pigs and chickens:  
434 Implication for gut health. *Front Vet Sci* 8:496.  
435 <https://doi.org/10.3389/fvets.2021.663727>
- 436 Chrystal P V, Moss AF, Khoddami A, Naranjo V, Selle P, Liu SY (2020) Effects of reduced  
437 crude protein levels, dietary electrolyte balance, and energy density on the performance  
438 of broiler chickens offered maize-based diets with evaluations of starch, protein, and  
439 amino acid metabolism. *Poult Sci* 99:1421–1431.  
440 <https://doi.org/https://doi.org/10.1016/j.psj.2019.10.060>
- 441 Clark DP, Pazdernik NJ, McGehee MR (2013) Chapter 13 - Protein Synthesis. In: Clark DP  
442 (ed) *Molecular Biology*, 2nd edn. Academic Cell, pp 369–416
- 443 Cox M, Nelson D (1942) *Lehninger principles of biochemistry*. 4th edn. W.H. Freeman and  
444 Company, New York. <https://doi.org/0.1002/bmb.2005.494033010419>
- 445 Cromwell GL (2000) Utilization of soy products in swine diets. In: Drackley JK (ed) *Soy in*  
446 *animal nutrition*. Fed. Anim. Sci. Soc., Savoy, IL, USA, pp 258-282
- 447 D’Mello J (2003) Amino acids as multifunctional molecules. In: D’Mello J (ed) *Amino acids*  
448 *in animal nutrition*, 2nd edn. CABI International, Wallingford, pp 1-14
- 449 Dai Z, Li X, Xi P, Zhang J, Wu G, Zhu W (2013a) L-glutamine regulates amino acid utilization  
450 by intestinal bacteria. *Amino Acids* 45 (3):501-512. <https://doi.org/10.1007/s00726-012-1264-4>
- 451

- 452 Dai Z, Wu Z, Yang Y, Wang J, Satterfield MC, Meininger CJ, Bazer FW, Wu G (2013b) Nitric  
453 oxide and energy metabolism in mammals. *Biofactors* 39 (4):383-391.  
454 <https://doi.org/10.1002/biof.1099>
- 455 Dangin M, Boirie Y, Garcia-Rodenas C, Gachon P, Fauquant J, Callier P, Ballèvre O, Beaufrère  
456 B (2001) The digestion rate of protein is an independent regulating factor of postprandial  
457 protein retention. *Am J Physiol Endocrinol Metab* 280 (2):E340-E348.  
458 <https://doi.org/10.1152/ajpendo.2001.280.2.E340>
- 459 Davis TA, Fiorotto ML, Burrin DG, Reeds PJ, Nguyen HV, Beckett PR, Vann RC, O'Connor  
460 PMJ (2002) Stimulation of protein synthesis by both insulin and amino acids is unique  
461 to skeletal muscle in neonatal pigs. *Am J Physiol Endocrinol Metab* 282 (4):E880-E890.  
462 <https://doi.org/10.1152/ajpendo.00517.2001>
- 463 De Feo P, Horber F, Haymond M (1992) Meal stimulation of albumin synthesis: A significant  
464 contributor to whole body protein synthesis in humans. *Am J Physiol* 263 (4 Pt 1):E794-  
465 799. <https://doi.org/10.1152/ajpendo.1992.263.4.E794>
- 466 Deng D, Yao K, Chu W, Li T, Huang R, Yin Y, Liu Z, Zhang J, Wu G (2009) Impaired  
467 translation initiation activation and reduced protein synthesis in weaned piglets fed a  
468 low-protein diet. *J Nutr Biochem* 20 (7):544-552.  
469 <https://doi.org/10.1016/j.jnutbio.2008.05.014>
- 470 Dieterich F, Boscolo W, Pacheco M, Silva V, Gonçalves G, Vidotti R (2014) Development and  
471 characterization of protein hydrolysates originated from animal agro industrial by-  
472 products. *J Dairy Vet Anim Res* 1 (2):00012.  
473 <https://doi.org/10.15406/jdvar.2014.01.00012>
- 474 Florou-Paneri P, Christaki E, Giannenas I, Bonos E, Skoufos I, Tsinas A, Tzora A, Peng J  
475 (2014) Alternative protein sources to soybean meal in pig diets. *J Food Agric Environ*  
476 12:655-660. <https://doi.org/10.1234/4.2014.5214>
- 477 Friedman M (1999) Chemistry, nutrition, and microbiology of d-amino acids. *J Agric Food*  
478 *Chem* 47 (9):3457-3479. <https://doi.org/10.1021/jf990080u>
- 479 Frikha M, Mohiti-Asli M, Chetrit C, Mateos G (2014) Hydrolyzed porcine mucosa in broiler  
480 diets: Effects on growth performance, nutrient retention, and histomorphology of the  
481 small intestine. *Poult Sci* 93 (2):400-411. <https://doi.org/10.3382/ps.2013-03376>
- 482 Garcia-Launay F, van der Werf H, Nguyen TTH, Le Tutour L, Dourmad JY (2014) Evaluation  
483 of the environmental implications of the incorporation of feed-use amino acids in pig  
484 production using life cycle assessment. *Livest Sci* 161:158-175.  
485 <https://doi.org/10.1016/j.livsci.2013.11.027>
- 486 Gilbert E, Wong E, Webb Jr K (2008) Board-invited review: Peptide absorption and utilization:  
487 Implications for animal nutrition and health. *J Anim Sci* 86 (9):2135-2155.  
488 <https://doi.org/10.2527/jas.2007-0826>
- 489 Gloaguen M, Le Floc'h N, Corrent E, Primot Y, van Milgen J (2014) The use of free amino  
490 acids allows formulating very low crude protein diets for piglets. *J Anim Sci* 92 (2):637-  
491 644. <https://doi.org/10.2527/jas.2013-6514>

- 492 Gottlob R, DeRouchey J, Tokach M, Goodband R, Dritz S, Nelssen J, Hastad C, Knabe D  
493 (2006) Amino acid and energy digestibility of protein sources for growing pigs. *J Anim*  
494 *Sci* 84 (6):1396-1402. <https://doi.org/10.2527/2006.8461396x>
- 495 Grazziotin A, Pimentel F, De Jong E, Brandelli A (2006) Nutritional improvement of feather  
496 protein by treatment with microbial keratinase. *Anim Feed Sci Technol* 126 (1-2):135-  
497 144. <https://doi.org/10.1016/j.anifeedsci.2005.06.002>
- 498 Guillet C, Zangarelli A, Gachon P, Morio Ba, Giraudet C, Rousset P, Boirie Y (2004) Whole  
499 body protein breakdown is less inhibited by insulin, but still responsive to amino acid,  
500 in nondiabetic elderly subjects. *J Clin Endocrinol Metab* 89 (12):6017-6024.  
501 <https://doi.org/10.1210/jc.2003-031323>
- 502 Han IK, Lee J (2000) The role of synthetic amino acids in monogastric animal production-  
503 review. *Asian-australas J Anim Sci* 13 (4):543-560.  
504 <https://doi.org/10.5713/ajas.2000.543>
- 505 Heimburger DC, Geels WJ, Bilbrey J, Redden DT, Keeney C (1997) Effects of small-peptide  
506 and whole protein enteral feedings on serum proteins and diarrhea in critically ill  
507 patients: A randomized trial. *J Parenter Enteral Nutr* 21 (3):162-167.  
508 <https://doi.org/10.1177/0148607197021003162>
- 509 Heo J, Kim J, Hansen C, Mullan B, Hampson D, Pluske J (2009) Feeding a diet with decreased  
510 protein content reduces indices of protein fermentation and the incidence of  
511 postweaning diarrhea in weaned pigs challenged with an enterotoxigenic strain of  
512 *Escherichia coli*. *J Anim Sci* 87 (9):2833-2843. <https://doi.org/10.2527/jas.2008-1274>
- 513 Hou Y, Wu Z, Dai Z, Wang G, Wu G (2017) Protein hydrolysates in animal nutrition: Industrial  
514 production, bioactive peptides, and functional significance. *J Anim Sci Biotechnol* 8  
515 (1):1-13. <https://doi.org/10.1186/s40104-017-0153-9>
- 516 Jeong JS, Park JW, Lee SI, Kim IH (2016) Apparent ileal digestibility of nutrients and amino  
517 acids in soybean meal, fish meal, spray-dried plasma protein and fermented soybean  
518 meal to weaned pigs. *Anim Sci J* 87 (5):697-702. <https://doi.org/10.1111/asj.12483>
- 519 Jones C, DeRouchey J, Nelssen J, Tokach M, Dritz S, Goodband R (2010) Effects of fermented  
520 soybean meal and specialty animal protein sources on nursery pig performance. *J Anim*  
521 *Sci* 88 (5):1725-1732. <https://doi.org/10.2527/jas.2009-2110>
- 522 Kerr BJ, Urriola PE, Jha R, Thomson JE, Curry SM, Shurson GC (2019) Amino acid  
523 composition and digestible amino acid content in animal protein by-product meals fed  
524 to growing pigs. *J Anim Sci* 97 (11), 4540-4547 <https://doi.org/10.1093/jas/skz294>
- 525 Kim S, Baker D, Easter R (2001) Dynamic ideal protein and limiting amino acids for lactating  
526 sows: The impact of amino acid mobilization. *J Anim Sci* 79 (9):2356-2366.  
527 <https://doi.org/10.2527/2001.7992356x>
- 528 Kodera T, Hara H, Nishimori Y, Nio N (2006) Amino acid absorption in portal blood after  
529 duodenal infusions of a soy protein hydrolysate prepared by a novel soybean protease  
530 d3. *J Food Sci* 71 (7):S517-S525. <https://doi.org/10.1111/j.1750-3841.2006.00118.x>

- 531 Kong F, Singh R (2008) Disintegration of solid foods in human stomach. *J Food Sci* 73 (5):R67-  
532 R80. <https://doi.org/10.1111/j.1750-3841.2008.00766.x>
- 533 Koopman R, Crombach N, Gijzen AP, Walrand S, Fauquant J, Kies AK, Lemosquet S, Saris  
534 WH, Boirie Y, van Loon LJ (2009) Ingestion of a protein hydrolysate is accompanied  
535 by an accelerated *in vivo* digestion and absorption rate when compared with its intact  
536 protein. *Am J Clin Nutr* 90 (1):106-115. <https://doi.org/10.3945/ajcn.2009.27474>
- 537 Krehbiel C, Matthews J (2003) Absorption of amino acids and peptides. In: D’Mello J (ed)  
538 Amino acids in animal nutrition, 2nd edn. CABI International, Wallingford, pp 41-70.  
539 <https://doi.org/10.1079/9780851996547.0041>
- 540 Kurauchi I, Yamane H, Tsuneyoshi Y, Denbow DM, Furuse M (2009) Central l-alanine reduces  
541 energy expenditure with a hypnotic effect under an acute stressful condition in neonatal  
542 chicks. *Amino Acids* 36 (1):131-135. <https://doi.org/10.1007/s00726-008-0042-9>
- 543 Le Floc’h N, Wessels A, Corrent E, Wu G, Bosi P (2018) The relevance of functional amino  
544 acids to support the health of growing pigs. *Anim Feed Sci Technol* 245:104-116.  
545 <https://doi.org/10.1016/j.anifeedsci.2018.09.007>
- 546 Li H, Wan H, Mercier Y, Zhang X, Wu C, Wu X, Tang L, Che L, Lin Y, Xu S (2014) Changes  
547 in plasma amino acid profiles, growth performance and intestinal antioxidant capacity  
548 of piglets following increased consumption of methionine as its hydroxy analogue. *Br J*  
549 *Nutr* 112 (6):855-867. <https://doi.org/10.1017/S000711451400172X>
- 550 Liao SF, Regmi N, Wu G (2018) Homeostatic regulation of plasma amino acid concentrations.  
551 *Front Biosci* 23 (2):640-655. <https://doi.org/10.2741/4610>
- 552 Lobley G (2003) Protein turnover—what does it mean for animal production? *Can J Anim Sci*  
553 83 (3):327-340. <https://doi.org/10.4141/A03-019>
- 554 López-Barrios L, Gutiérrez-Urbe JA, Serna-Saldívar SO (2014) Bioactive peptides and  
555 hydrolysates from pulses and their potential use as functional ingredients. *J Food Sci* 79  
556 (3):R273-R283. <https://doi.org/10.1111/1750-3841.12365>
- 557 Martínez-Alvarez O, Chamorro S, Brenes A (2015) Protein hydrolysates from animal  
558 processing by-products as a source of bioactive molecules with interest in animal  
559 feeding: A review. *Food Res Int* 73:204-212.  
560 <https://doi.org/10.1016/j.foodres.2015.04.005>
- 561 Min B, Hong J, Kwon O, Lee W, Kim Y, Kim I, Cho W, Kim J (2004) The effect of feeding  
562 processed soy protein on the growth performance and apparent ileal digestibility in  
563 weanling pigs. *Asian-australas J Anim Sci* 17 (9):1271-1276.  
564 <https://doi.org/10.5713/ajas.2004.1271>
- 565 Morales A, Buenabad L, Castillo G, Espinoza, S, Arce N, Bernal H, Htoo JK Cervantes, M  
566 (2020) Serum concentration of free amino acids in pigs of similar performance fed diets  
567 containing protein-bound or protein-bound combined with free amino acids. *Anim Feed*  
568 *Sci Technol* 267:114552.  
569 <https://doi.org/https://doi.org/10.1016/j.anifeedsci.2020.114552>

- 570 Morales A, Buenabad L, Castillo G, Vázquez L, Espinoza S, Htoo J, Cervantes M (2017)  
571 Dietary levels of protein and free amino acids affect pancreatic proteases activities,  
572 amino acids transporters expression and serum amino acid concentrations in starter pigs.  
573 *J Anim Physiol Anim Nutr* 101 (4):723-732. <https://doi.org/10.1111/jpn.12515>
- 574 Munro HN (2012) *Mammalian protein metabolism*, vol 4. Academic Press, New York and  
575 London.
- 576 Nørgaard JV, Blaabjerg K, Poulsen HD (2012) Salmon protein hydrolysate as a protein source  
577 in feed for young pigs. *Anim Feed Sci Technol* 177 (1-2):124-129.  
578 <https://doi.org/10.1016/j.anifeedsci.2012.08.003>
- 579 NRC (2012) *Nutrient requirements of swine: Eleventh revised edition*. The National Academies  
580 Press, Washington, DC. <https://doi.org/doi:10.17226/13298>
- 581 Nyachoti C, Lange CD, McBride B, Schulze H (1997) Significance of endogenous gut nitrogen  
582 losses in the nutrition of growing pigs: A review. *Can J Anim Sci* 77 (1):149-163.  
583 <https://doi.org/10.4141/A96-044>
- 584 O'Connor PM, Bush JA, Suryawan A, Nguyen HV, Davis TA (2003) Insulin and amino acids  
585 independently stimulate skeletal muscle protein synthesis in neonatal pigs. *Am J Physiol*  
586 *Endocrinol Metab* 284 (1):E110-E119. <https://doi.org/10.1152/ajpendo.00326.2002>
- 587 Paddon-Jones D, Sheffield-Moore M, Urban RJ, Sanford AP, Aarsland A, Wolfe RR, Ferrando  
588 AA (2004) Essential amino acid and carbohydrate supplementation ameliorates muscle  
589 protein loss in humans during 28 days bedrest. *J Clin Endocrinol Metab* 89 (9):4351-  
590 4358. <https://doi.org/10.1210/jc.2003-032159>
- 591 Papadopoulos M, El Boushy A, Roodbeen A, Ketelaars E (1986) Effects of processing time  
592 and moisture content on amino acid composition and nitrogen characteristics of feather  
593 meal. *Anim Feed Sci Technol* 14 (3-4):279-290. [https://doi.org/10.1016/0377-  
594 8401\(86\)90100-8](https://doi.org/10.1016/0377-8401(86)90100-8)
- 595 Pasupuleti VK, Braun S (2008) State of the art manufacturing of protein hydrolysates. In:  
596 Pasupuleti VK (ed) *Protein hydrolysates in biotechnology*. Springer, pp 11-32.  
597 [https://doi.org/10.1007/978-1-4020-6674-0\\_2](https://doi.org/10.1007/978-1-4020-6674-0_2)
- 598 Pasupuleti VK, Holmes C, Demain AL (2008) Applications of protein hydrolysates in  
599 biotechnology. In: Pasupuleti VK (ed) *Protein hydrolysates in biotechnology*. Springer,  
600 pp 1-9. [https://doi.org/10.1007/978-1-4020-6674-0\\_1](https://doi.org/10.1007/978-1-4020-6674-0_1)
- 601 Poudel P, Levesque CL, Samuel R, St-Pierre B (2020) Dietary inclusion of peptiva, a peptide-  
602 based feed additive, can accelerate the maturation of the fecal bacterial microbiome in  
603 weaned pigs. *BMC Vet Res* 16 (1):1-13. <https://doi.org/10.1186/s12917-020-02282-x>
- 604 Reidy PT, Walker DK, Dickinson JM, Gundermann DM, Drummond MJ, Timmerman KL, Fry  
605 CS, Borack MS, Cope MB, Mukherjea R (2013) Protein blend ingestion following  
606 resistance exercise promotes human muscle protein synthesis. *J Nutr* 143 (4):410-416.  
607 <https://doi.org/10.3945/jn.112.168021>
- 608 Riedijk MA, Stoll B, Chacko S, Schierbeek H, Sunehag AL, van Goudoever JB, Burrin DG  
609 (2007) Methionine transmethylation and transsulfuration in the piglet gastrointestinal



- 610 tract. Proceedings of the National Academy of Sciences 104 (9):3408-3413.  
611 <https://doi.org/10.1073/pnas.0607965104>
- 612 Sauer WC, Ozimek L (1986) Digestibility of amino acids in swine: Results and their practical  
613 applications. A review. Livest Prod Sci 15 (4):367-388.
- 614 Souffrant W, Darcy-Vrillon B, Corring T, Laplace J, Köhler R, Gebhardt G, Rerat A (1986)  
615 Recycling of endogenous nitrogen in the pig (preliminary results of a collaborative  
616 study). Arch Anim Nutr 36 (2-3):269-274. <https://doi.org/10.1080/17450398609425271>
- 617 Stoll B, Burrin D (2006) Measuring splanchnic amino acid metabolism *in vivo* using stable  
618 isotopic tracers. J Anim Sci 84:E60-E72. [https://doi.org/10.2527/2006.8413\\_supplE60x](https://doi.org/10.2527/2006.8413_supplE60x)
- 619 Ten Have GA, Engelen MP, Luiking YC, Deutz NE (2007) Absorption kinetics of amino acids,  
620 peptides, and intact proteins. Int J Sport Nutr Exerc Metab 17 (s1):S23-S36.  
621 <https://doi.org/10.1123/ijsnem.17.s1.s23>
- 622 Trevisi P, Priori D, Jansman AJ, Luise D, Koopmans SJ, Hynönen U, Palva A, Van Der Meulen  
623 J, Bosi P (2018) Molecular networks affected by neonatal microbial colonization in  
624 porcine jejunum, luminally perfused with enterotoxigenic *Escherichia coli*, f4ac fimbria  
625 or *Lactobacillus amylovorus*. PloS One 13 (8):e0202160.  
626 <https://doi.org/10.1371/journal.pone.0202160>
- 627 Tuitoek K, Young L, De Lange C, Kerr B (1997) The effect of reducing excess dietary amino  
628 acids on growing-finishing pig performance: An elevation of the ideal protein concept.  
629 J Anim Sci 75 (6):1575-1583. <https://doi.org/10.2527/1997.7561575x>
- 630 van der Peet-Schwering C, van Krimpen M, Kemme P, Binnendijk G, van Diepen J, Jongbloed  
631 A, Henniphof C (2006) Alternative protein crops in diets of organically housed weanling  
632 pigs. PraktijkRapport Varkens 47. Animal Sciences Group,
- 633 van Loon LJ, Saris WH, Verhagen H, Wagenmakers AJ (2000) Plasma insulin responses after  
634 ingestion of different amino acid or protein mixtures with carbohydrate. Am J Clin Nutr  
635 72 (1):96-105. <https://doi.org/10.1093/ajcn/72.1.96>
- 636 van Milgen J, Dourmad J-Y (2015) Concept and application of ideal protein for pigs. J Anim  
637 Sci Biotechnol 6 (1):15. <https://doi.org/10.1186/s40104-015-0016-1>
- 638 Wang B, Mi M, Zhang Q, Bao N, Pan L, Zhao Y, Qin G (2021a) Relationship between the  
639 amino acid release kinetics of feed proteins and nitrogen balance in finishing pigs.  
640 Animal 15 (10):100359. <https://doi.org/j.animal.2021.100359>
- 641 Wang S, Zeng X, Yang Q, Qiao S (2016) Antimicrobial peptides as potential alternatives to  
642 antibiotics in food animal industry. Int J Mol Sci 17 (5):603
- 643 Wang T, Osho SO, Adeola O (2018) Additivity of apparent and standardized ileal digestibility  
644 of amino acid determined by chromic oxide and titanium dioxide in mixed diets  
645 containing wheat and multiple protein sources fed to growing pigs. J Anim Sci 96  
646 (11):4731-4742. <https://doi.org/10.1093/jas/sky326>
- 647 Wang WW, Feng QQ, Wang J, Wu SG, Qi GH, Zhang HJ (2021b) Cyst(e)ine fortification in  
648 low crude protein diet improves growth performance of broilers by modulating serum  
649 metabolite profile. J Proteom 238:104154. <https://doi.org/10.1016/j.jprot.2021.104154>

- 650 Wu G (1998) Intestinal mucosal amino acid catabolism. *J Nutr* 128 (8):1249-1252.  
651 <https://doi.org/10.1093/jn/128.8.1249>
- 652 Wu G (2010) Functional amino acids in growth, reproduction, and health. *Adv Nutr* 1 (1):31-  
653 37. <https://doi.org/10.3945/an.110.1008>
- 654 Wu G (2013a) *Amino acids: Biochemistry and nutrition*. CRC Press, Boca Raton.  
655 <https://doi.org/10.1201/b14661>
- 656 Wu G (2013b) Functional amino acids in nutrition and health. *Amino Acids* 45 (3):407-411.  
657 <https://doi.org/10.1007/s00726-013-1500-6>
- 658 Wu G, Bazer FW, Dai Z, Li D, Wang J, Wu Z (2014) Amino acid nutrition in animals: Protein  
659 synthesis and beyond. *Annu Rev Anim Biosci* 2 (1):387-417.  
660 <https://doi.org/10.1146/annurev-animal-022513-114113>
- 661 Wu G, Bazer FW, Davis TA, Kim SW, Li P, Rhoads JM, Satterfield MC, Smith SB, Spencer  
662 TE, Yin Y (2009) Arginine metabolism and nutrition in growth, health and disease.  
663 *Amino Acids* 37 (1):153-168. <https://doi.org/10.1007/s00726-008-0210-y>
- 664 Wu G, Wu Z, Dai Z, Yang Y, Wang W, Liu C, Wang B, Wang J, Yin Y (2013) Dietary  
665 requirements of “nutritionally non-essential amino acids” by animals and humans.  
666 *Amino Acids* 44 (4):1107-1113. <https://doi.org/10.1007/s00726-012-1444-2>
- 667 Yen JT, Kerr B, Easter R, Parkhurst A (2004) Difference in rates of net portal absorption  
668 between crystalline and protein-bound lysine and threonine in growing pigs fed once  
669 daily. *J Anim Sci* 82 (4):1079-1090. <https://doi.org/10.1093/ansci/82.4.1079>
- 670 Zhang L, Piao X (2021) Different dietary protein sources influence growth performance,  
671 antioxidant capacity, immunity, fecal microbiota and metabolites in weaned piglets.  
672 *Anim Nutr*. <https://doi.org/https://doi.org/10.1016/j.aninu.2021.06.013>
- 673 Zhang S, Qiao S, Ren M, Zeng X, Ma X, Wu Z, Thacker P, Wu G (2013) Supplementation with  
674 branched-chain amino acids to a low-protein diet regulates intestinal expression of  
675 amino acid and peptide transporters in weanling pigs. *Amino Acids* 45 (5):1191-1205.  
676 <https://doi.org/10.1007/s00726-013-1577-y>

677

## TABLES AND FIGURES

678 Table 1. Protein and amino acid digestibility (%) of different protein sources fed to monogastric  
679 farm animals.<sup>a,b</sup>

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

680 <sup>a</sup> Summary of the ileal digestibility of some dietary protein sources. Free amino acids are not included in the table as they are  
681 considered to be completely digestible682 <sup>b</sup> Values are presented as percentage for crude protein (CP), lysine (LYS), methionine (MET), and total amino acids (TAA).683 <sup>c</sup> Apparent ileal digestible values – measured by the proportion of ingested AA left in the distal ileum684 <sup>d</sup> Standardized ileal digestible values - Apparent ileal digestibility corrected for endogenous losses

685

686 Table 2. Nutritional classification of proteinogenic amino acids in the diets of monogastric farm  
687 animals.<sup>a</sup>

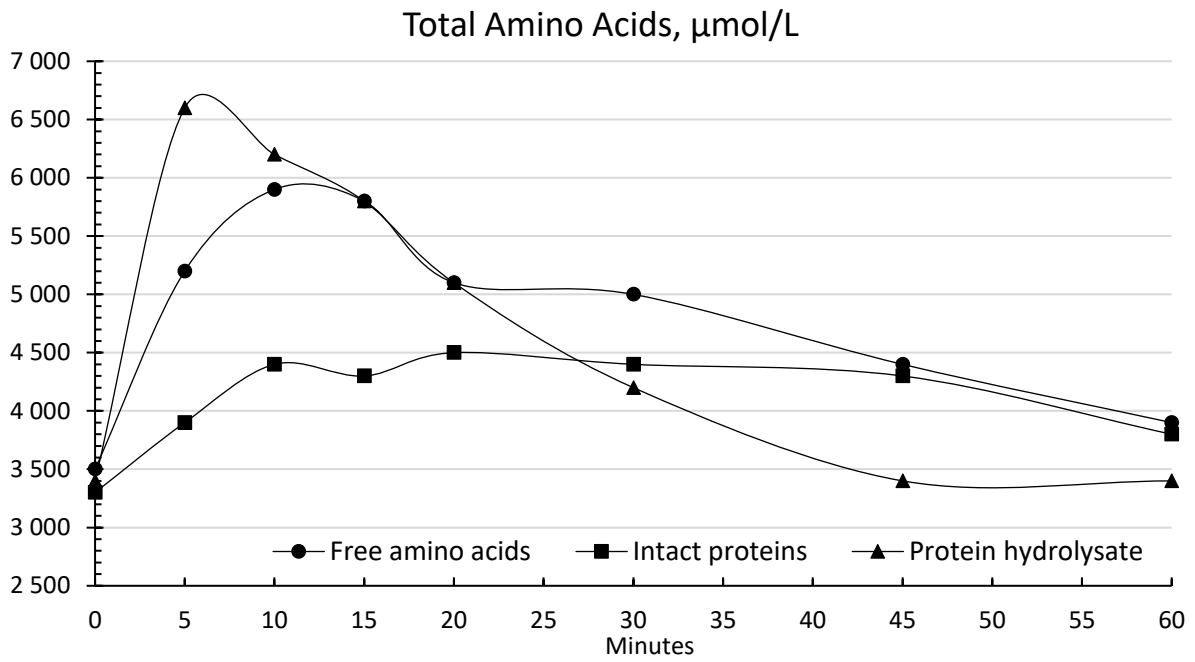
Mammals <sup>b</sup>			Poultry <sup>c</sup>		
EAA	CEAA	NEAA	EAA	CEAA	NEAA
Cys	Gln	Ala	Arg	Gln	Ala
His	Glu	Asn	Cys	Glu	Asn
Ile	Gly	Asp	Gly	Cys <sup>e</sup>	Asp
Leu	Pro	Ser	His	Tyr <sup>f</sup>	Ser
Lys	Arg <sup>d</sup>		Ile		
Met	Cys <sup>e</sup>		Leu		
Phe	Tyr <sup>f</sup>		Lys		
Thr			Met		
Trp			Phe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

688 <sup>a</sup> Table derived from Wu *et al.* (2014). Amino acids are listed in their three-letter IUPAC abbreviation. EAA – Essential amino  
689 acids, CEAA – Conditionally essential amino acids; NEAA – Non-essential amino acids690 <sup>b</sup> Includes pigs and ruminants, also applicable for rodents691 <sup>c</sup> Includes chickens, ducks, quails, and geese692 <sup>d</sup> Arginine is classified as a conditionally essential amino acid for pig by NRC (2012)693 <sup>e</sup> Can be synthesized in the liver by conversion of methionine and serine, but methionine needs to be in sufficient amount694 <sup>f</sup> Can be synthesized in the liver by conversion of phenylalanine as long as it is in sufficient amounts

695

696

697



698

699

700

701

702

703

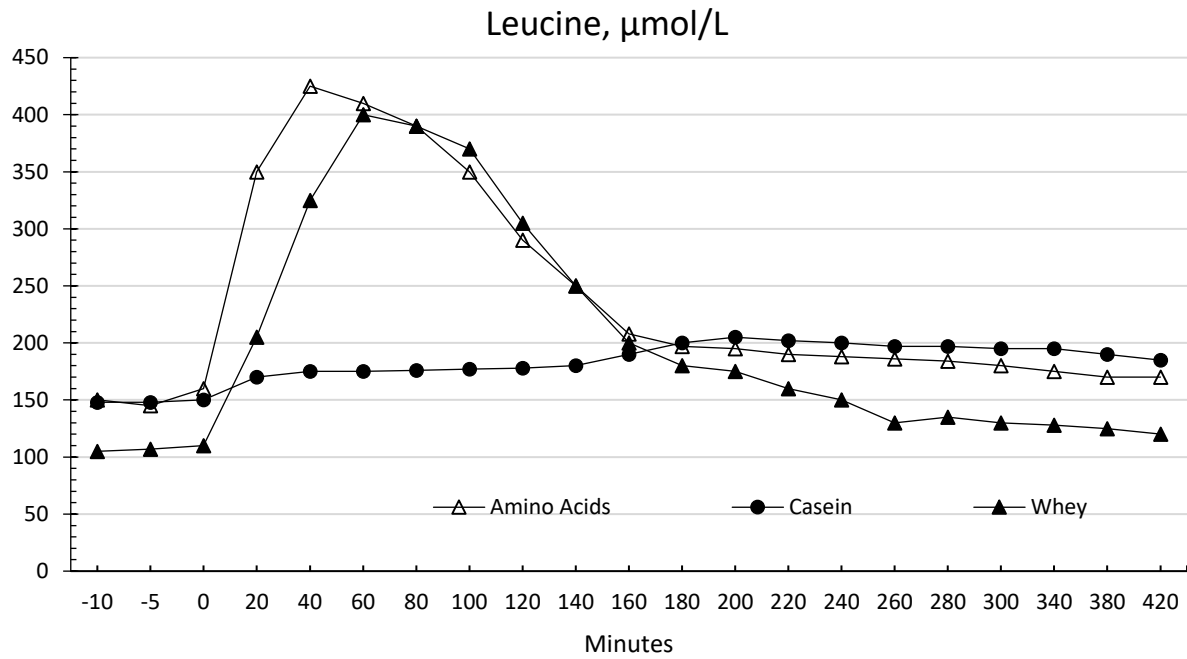
704

705

706

707

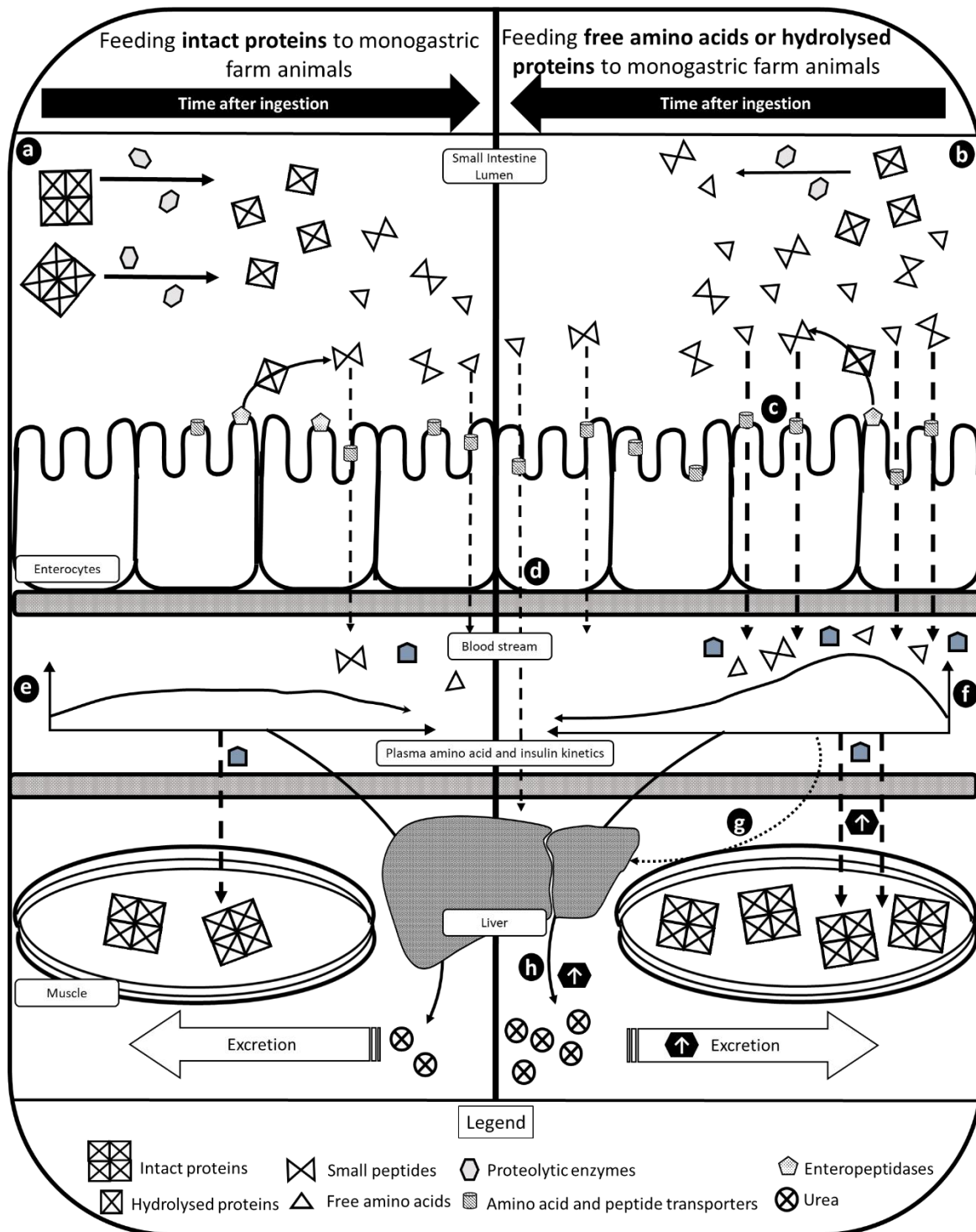
**Fig. 1** Plasma concentrations of total proteinogenic amino acids ( $\mu\text{mol/L}$ ) in the portal vein of rats after gastric infusion of intact protein ( $\blacksquare$ ), hydrolysate ( $\blacktriangle$ ), or free amino acids ( $\bullet$ ) with the same amino acid profile (Kodera et al. 2006)



708

709 **Fig. 2** Postprandial leucine plasma concentration ( $\mu\text{mol/L}$ ) of healthy young men given free  
 710 amino acids ( $\Delta$ ), whey protein ( $\blacktriangle$ ), and casein ( $\bullet$ ) protein sources with identical amino acid  
 711 profile (Dangin et al. 2001)  
 712

713



714

715

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

724 in the proximal part of the small intestine (c) although with this increased substrate  
725 availability, amino acids may be directed to the liver for deamination/catabolism (d). Since  
726 protein digestion takes time, the appearance of its products in the plasma is more constant  
727 resulting in a characteristically flat kinetics of amino acids after feeding intact proteins (e).  
728 Feeding hydrolysates and free amino acids induces a quicker appearance of plasma amino  
729 acids and an asymmetric distribution of postprandial plasma amino acids kinetics that is  
730 skewed towards the beginning (f). Plasma amino acids induces the release of insulin that  
731 increases the uptake of amino acids by the muscle and increase protein synthesis. However, in  
732 this postprandial state, due to the rapid appearance of amino acids in the plasma after feeding  
733 hydrolysates or free amino acids, there is a period of temporary imbalance of amino acid  
734 uptake of sites of protein synthesis, like the muscle, that causes more amino acids to be  
735 catabolized compared to feeding intact proteins (g). As a consequence to this increased rate of  
736 amino acid catabolism, more urea is produced by the liver which can also be interpreted as a  
737 lower efficiency of use of dietary nitrogen (h)  
738

739