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

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

2

3 FA Eugenio^{1,2}, J van Milgen¹, J Duperray², R Sergheraert², N Le Floch^{h1,3}

4 ¹PEGASE, INRAE, Institut Agro, 35590, Saint Gilles, France

5 ²BCF Life Sciences, Boisel, 56140 Pleucadeuc, France

6 ³Corresponding author: 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.

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TABLES AND FIGURES

678 Table 1. Protein and amino acid digestibility (%) of different protein sources fed to monogastric
679 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

680 ^a 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 ^b Values are presented as percentage for crude protein (CP), lysine (LYS), methionine (MET), and total amino acids (TAA).683 ^c Apparent ileal digestible values – measured by the proportion of ingested AA left in the distal ileum684 ^d Standardized ileal digestible values - Apparent ileal digestibility corrected for endogenous losses

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686 Table 2. Nutritional classification of proteinogenic amino acids in the diets of monogastric farm
687 animals.^a

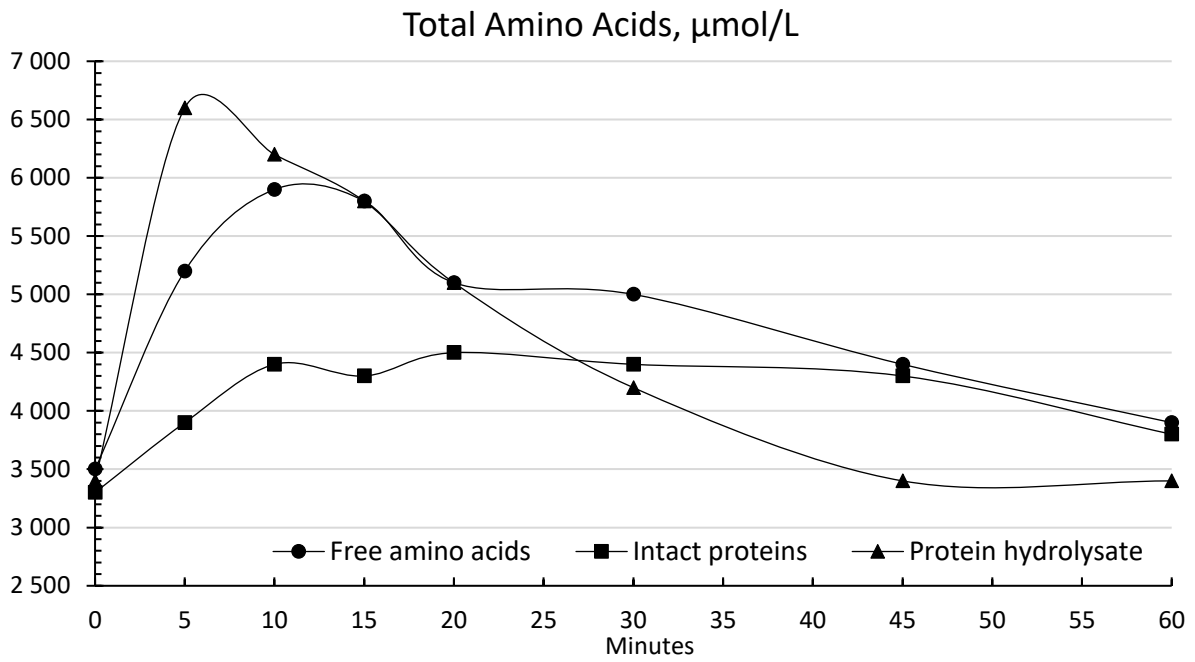
Mammals ^b			Poultry ^c		
EAA	CEAA	NEAA	EAA	CEAA	NEAA
Cys	Gln	Ala	Arg	Gln	Ala
His	Glu	Asn	Cys	Glu	Asn
Ile	Gly	Asp	Gly	Cys ^e	Asp
Leu	Pro	Ser	His	Tyr ^f	Ser
Lys	Arg ^d		Ile		
Met	Cys ^e		Leu		
Phe	Tyr ^f		Lys		
Thr			Met		
Trp			Phe		
Tyr			Pro		
Val			Thr		
			Trp		
			Tyr		
			Val		

688 ^a 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 ^b Includes pigs and ruminants, also applicable for rodents691 ^c Includes chickens, ducks, quails, and geese692 ^d Arginine is classified as a conditionally essential amino acid for pig by NRC (2012)693 ^e Can be synthesized in the liver by conversion of methionine and serine, but methionine needs to be in sufficient amount694 ^f Can be synthesized in the liver by conversion of phenylalanine as long as it is in sufficient amounts

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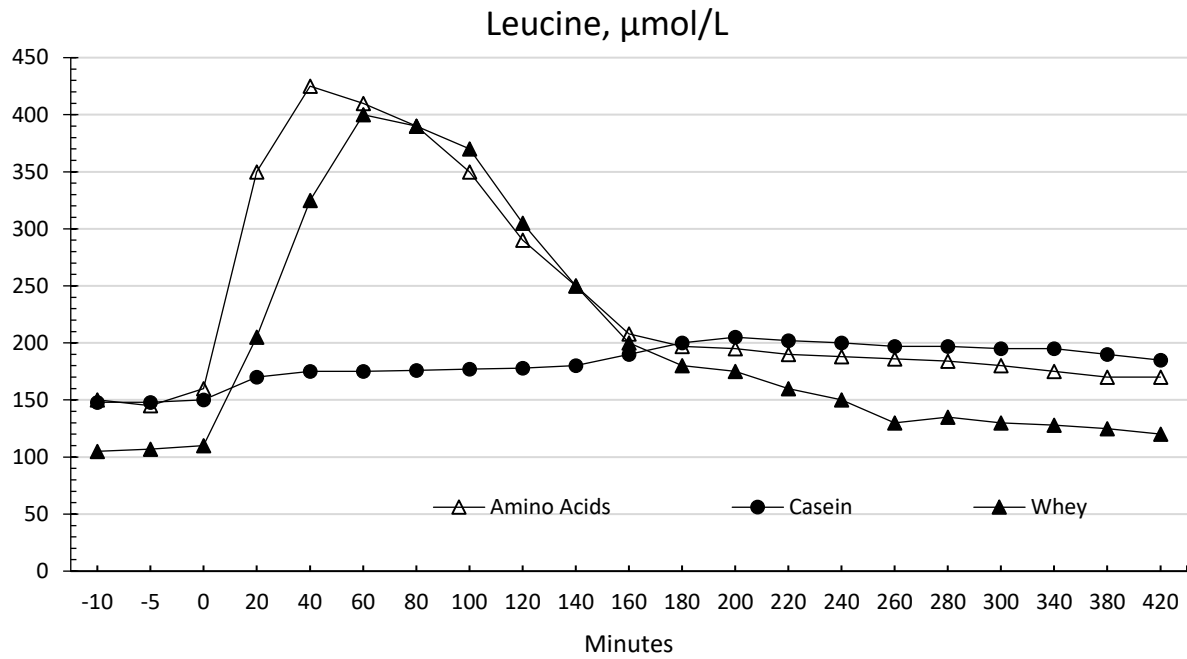
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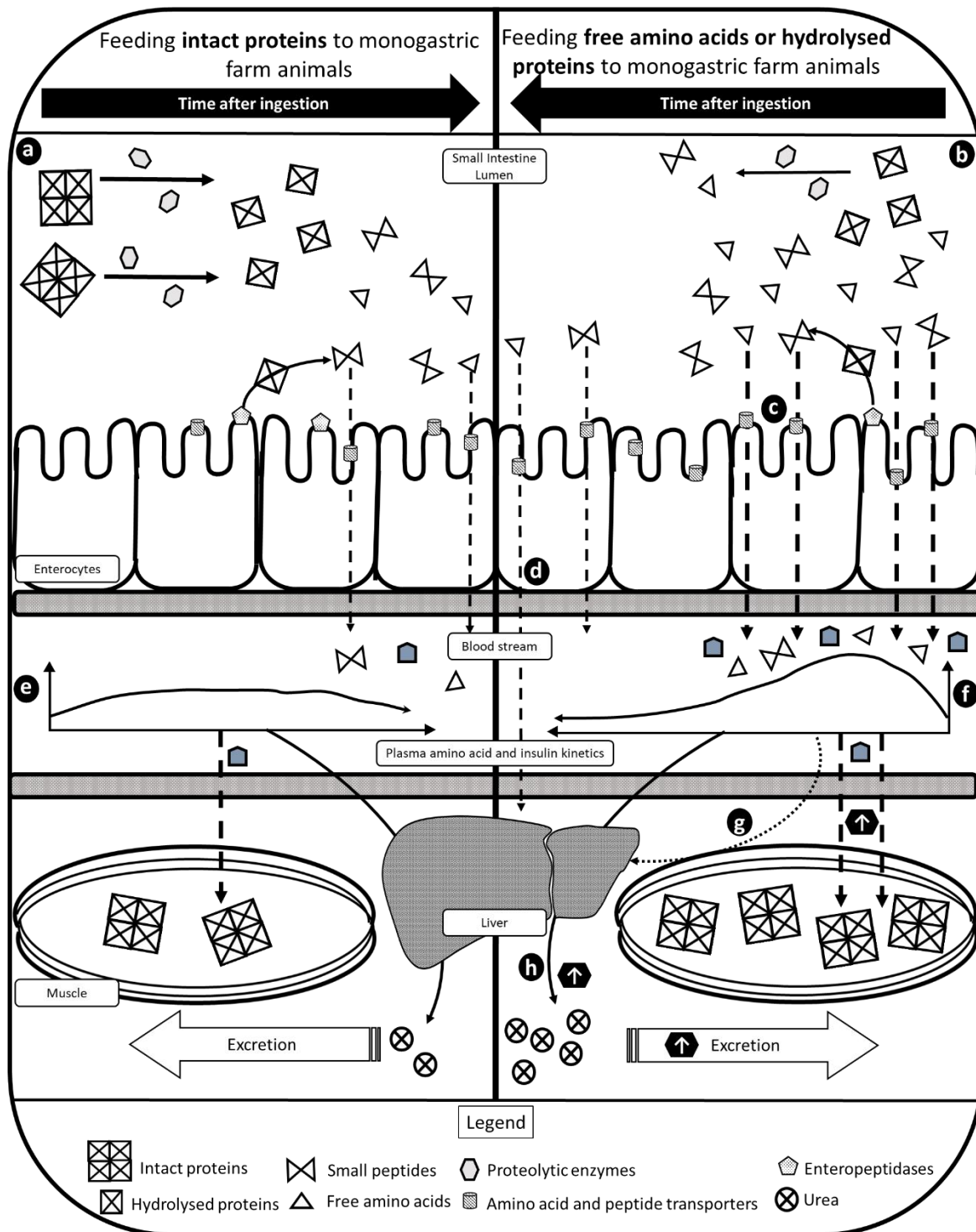
Fig. 1 Plasma concentrations of total proteinogenic amino acids ($\mu\text{mol/L}$) in the portal vein of rats after gastric infusion of intact protein (\blacksquare), hydrolysate (\blacktriangle), or free amino acids (\bullet) with the same amino acid profile (Kodera et al. 2006)



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

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)
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