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ABSTRACT

Dietary proteins need to be digested first while free amino acids (**AAs**) and small peptides are readily available for absorption and rapidly appear in the blood. The rapid postprandial appearance of dietary AA in the systemic circulation may result in inefficient AA utilisation for protein synthesis of peripheral tissues if other nutrients implicated in AA and protein metabolism are not available at the same time. The objective of this experiment was to compare the postprandial concentrations of plasma AA and other metabolites after the ingestion of a diet that provided AA either as proteins or as free AA and small peptides. Twenty-four male growing pigs (38.8 ± 2.67 kg) fitted with a jugular catheter were assigned to one of three diets that provided AA either in protein form (**INT**), free AA and small peptides (**HYD**), or as free AA (**FAA**). After an overnight fast and initial blood sampling, a small meal was given to each pig followed by serial blood collection for 360 min. Postprandial concentrations of plasma AA, glucose, insulin, and urea were then measured from the collected blood. Non-linear regression was used to summarise the postprandial plasma AA kinetics. Fasting concentrations of urea and some AA were higher ($P < 0.05$) while postprandial plasma insulin and glucose were lower ($P < 0.01$) for INT than for HYD and FAA. The area under the curve of plasma concentration after meal distribution was lower for INT for most AAs ($P < 0.05$), resulting in a flatter curve compared to HYD and FAA. This was the result of the slower appearance of dietary AA in the plasma when proteins are fed instead of free AA and small peptides. The flatter curve may also result from more AAs being metabolised by the intestine and liver when INT was fed. The metabolism of AA of the intestine and liver was higher for HYD than FAA. Providing AA as proteins or as free AA and small peptides affected the postprandial plasma kinetics of AA, urea, insulin, and glucose. Whether the flat kinetics when feeding proteins has a positive or negative effect on AA metabolism still needs to be explored.

Implications

To increase the efficiency of nitrogen utilisation and reduce nitrogen excretion, free amino acids are increasingly used in diets of monogastric animals. Feeding diets with free amino acids and small peptides resulted in lower first-pass amino acid metabolism in the intestine and liver compared to providing these amino acids as proteins, suggesting a more efficient use of the amino acid. However, it remains to be elucidated if this lower first-pass metabolism is accompanied by an overall increase in the efficiency of amino acid use.

Introduction

Using free, non-bound amino acids (**AAs**) in animal diets instead of traditional protein sources like soybean meal is a way to address increasing concerns regarding the negative environmental impact of animal husbandry (Garcia-Launay et al., 2014). Apart from the reduction of the dependency on protein sources that are resource-intensive, an alternative is to use by-products of animal production as they contribute to a circular economy and the lessening of wastes (Garcia-Launay et al., 2014). Furthermore, using free AA allows for formulating diets with a reduced CP content with an AA profile close to the requirement, an approach that reduces nitrogen excretion (Chrystal et al., 2020). Even though providing low CP diets with supplemental free AA has been a well-established practice in animal nutrition (Gloaguen et al., 2014),

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some studies reported that feeding animals with diets with large amounts of free AA reduces the efficiency of AA utilisation for growth (Batterham and Bayley, 1989; Yen et al., 2004). Since free AA and small peptides can be absorbed directly in the small intestine without the need for digestive hydrolysis (Wu, 1998; Krehbiel and Matthews, 2003), they appear rapidly in the plasma. Differences in the rate of appearance between AA, other nutrients, or energy may cause a temporary imbalance at the sites of protein synthesis and result in increased AA catabolism compared with feeding AA as proteins (Bos et al., 2003; Yen et al., 2004).

The efficiency by which dietary AA can be used for protein synthesis depends on both the form and balance of dietary AA relative to the requirement. In a previous experiment, the effect of the AA balance on their utilisation was studied (Eugenio et al., 2022). In this experiment, the focus is on the form by which AAs are supplied, using feather proteins processed in varying degrees as a model protein source. Presently, feathers are mainly valorised in the animal feed sector as feather meal by simple physical processing through steam and pressure that leaves the protein intact but makes it accessible to digestive enzymes. A more intensive process like extensive acid hydrolysis transforms the protein fraction of feathers into free AA and small peptides (Kersanté et al., 2021). The pig is a well-recognised animal model for metabolic studies, and their size makes serial blood sampling possible. In this study, we compared the postprandial plasma concentrations of AA, metabolites, and insulin in pigs fed either feather meal, extensively hydrolysed feathers composed of 84% of free AA and 16% small peptides, or a mix of free AA following the AA profile of the extensively hydrolysed feathers. We hypothesised that pigs fed a diet with proteins have lower postprandial plasma concentrations of AA and insulin compared to pigs fed a diet with the extensively hydrolysed feather or the mix of free AA.

Material and methods

Animal housing, diets, and experimental design

The experiment was carried out at UE3P (INRAE, Saint-Gilles, France https://doi.org/10.15454/1.5573932732039927E12). Thirty male Piétrain × Large-White × Landrace growing pigs were selected at 12 weeks of age (33.7 ± 4.1 kg). They were surgically fitted with a jugular catheter following the procedure of Eugenio et al. (2022). Throughout the experiment, pigs were housed in individual pens (1.0 × 2.0 m) equipped with a nipple drinker and a feeding trough. The room was environmentally controlled with temperature maintained at 26 °C, relative humidity at 60%, and 12 h of light daily. After the surgery, pigs were allotted to one of three dietary treatments where AAs were provided as feather meal as a model of proteins (**INT**), as extensively hydrolysed feathers providing AA as free AA and small peptides (**HYD**), or as purified free AA with the same AA profile as that of HYD (**FAA**). The ingredient and nutrient composition of the experimental diets are given in Table 1. Apart from the AA source, the ingredient composition of the three diets was almost identical. L-Trp was added in HYD (and also in FAA) as it is destroyed by the extensive acid hydrolysis process. All free AAs were bought from Sigma Aldrich Chimie (St. Quentin Fallavier, France) except for L-Cys and L-Tyr, which were supplied by BCF Life Sciences (Pleucadeuc, France). Experimental diets were offered as mash. A daily feed allowance of around 1 200 g/day was distributed twice daily as meals (morning and afternoon). Throughout the experiment, the pigs were offered a mixture of the experimental diet (at 90%) and a regular, pelleted starter diet (at 10%) as the pigs did not eat well when fed exclusively with any of the experimental diets.

Table 1
Composition and nutrient analysis (as fed) of the three experimental diets fed to growing pigs.[1]

Item	INT	HYD	FAA
Ingredients, %			
Limestone	0.90	0.90	0.90
Monocalcium phosphate	2.20	2.20	2.20
Vitamin and mineral premix[2]	0.50	0.50	0.50
Refined iodised salt	0.60	0.60	0.60
Sugar	2.00	2.00	2.00
Extensively hydrolysed feathers		17.0	
Feather meal	17.0		
Vegetable oil	4.00	4.00	4.00
Corn starch	67.8	67.7	69.7
Synthetic fibre	5.00	5.00	5.00
L-Trp		0.10	0.10
Amino acid mix[3]			15.0
Analysed content			
Moisture, %	10.3	11.1	9.66
Total nitrogen, %	2.58	2.28	2.10
Gross energy, MJ/kg	16.6	15.8	16.4
Total amino acids, g/kg	143	150	136
Amino acids, g/kg			
Ala	7.40	6.70	5.40
Arg	9.50	8.40	7.60
Asp	9.80	9.60	9.40
Cys	4.80	12.2	12.2
Glu	15.8	14.9	13.1
Gly	11.8	11.1	9.70
His	0.90	1.00	0.50
Ile	7.20	7.10	6.60
Leu	12.3	13.4	11.4
Val	11.7	11.4	10.3
Lys	2.60	2.50	3.10
Met	0.60	0.80	0.80
Phe	7.30	7.40	7.40
Pro	13.6	13.4	13.2
Ser	16.9	17.9	14.1
Thr	6.60	6.70	5.20
Tyr	0.90	0.70	0.80
Trp	3.50	5.10	4.90

Abbreviations: INT = intact protein diet; HYD = extensively hydrolysed protein diet; FAA = free amino acid diet

[1] Experimental diets were offered to the pigs as mash.

[2] Provided per kg of complete diet: vitamin A, 1 000 000 IU; vitamin D3, 200 000 IU; vitamin E, 4 000 mg; vitamin B1, 400 mg; vitamin B2, 800 mg; calcium pantothenate, 2 170 mg; niacin, 3 000 mg; vitamin B12, 4 mg; vitamin B6, 200 mg; vitamin K3, 400 mg; folic acid, 200 mg; biotin, 40 mg; choline chloride, 100 000 mg; iron (sulphate), 11 200 mg; iron (carbonate), 4 800 mg; copper (sulphate), 2 000 mg; zinc (oxide), 20 000 mg; manganese (oxide), 8 000 mg; iodine (iodate), 40 mg; cobalt (carbonate), 20 mg; and selenium (selenite), 30 mg.

[3] Amino acid mix consisted of L-Lys, DL-Met, L-Thr, L-Val, L-Arg, L-His, L-Ile, L-Leu, L-Glu, L-Tyr, L-Asp, L-Gly, L-Ala, L-Cys, L-Phe, L-Pro, and L-Ser.

Meal tests and serial blood sampling

One week after catheter implantation, pigs were submitted to a meal test that consisted of giving each pig a small meal followed by serial blood collection. After an overnight fast, a first blood sample (4–5 mL) was collected. Then, a small meal (300 g) was given to ensure the pigs ate all the offered experimental diets in less than 10 min. From 10 to 360 min after the meal, blood was collected at 10, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, 240, and 360 min. Blood was collected in tubes lined with heparin and immediately placed on an ice bed before being centrifuged (3 000*g* for 10 min at 4 °C) to separate plasma. Plasma samples were then stored in freezers (−80 or −20 °C) until further analyses. The catheters were rinsed with sterile saline after each blood sampling. During the blood sampling procedure, pigs had no access to feed but had access to water. This procedure was done for 24 pigs

(eight pigs per treatment). The other pigs were kept for the duration of the experiment to serve as a replacement in the event of health problems or malfunctioning of the catheters.

Feed analyses

The nitrogen concentration in the feed was analysed by combustion in an automated analyser (LECO FP-428 Nitrogen Determinator, LECO, St. Joseph, MI). Following the standard method for AA analysis of feedstuff (AOAC, 2005), feed samples were hydrolysed in 6 N HCl for 24 h at 110 °C with constant agitation to release AA. For Met and Cys analysis, oxidation with performic acid was done for 4 h before hydrolysis for 18 h. The Trp content was determined after alkaline hydrolysis with barium hydroxide for 16 h at 120 °C. The AA content was analysed after derivatisation by AccQtag (AccQ-Fluor reagent kit, Waters, Guyancourt, France) using an HPLC system (Alliance System 2690, Waters, Guyancourt, France). The gross energy content was measured in an adiabatic bomb calorimeter (IKA, Staufen, Germany).

Plasma amino acids, metabolites, and insulin assays

The plasma concentrations of AA were analysed using a UPLC system (Waters acquity ultra performance liquid chromatography system, Waters, Guyancourt, France) with the MassTrak AAA column after derivatisation of samples using the AccQtag Ultra method (Waters, Milford, MA, USA). Plasma glucose (Glucose (HK), Thermo Fisher Scientific, Vantaa, Finland) and urea (Urea kit, Thermo Fisher Scientific, Asnieres-Sur-Seine, France) were analysed with commercial kits using colorimetric methods in an automatic analyser, Konelab 20i (Thermo fisher scientific, Courtaboeuf, France). Plasma concentrations of insulin were measured using a radioimmunoassay kit (Insulin-CT, CisBio Bioassays, Codolet, France) and an AIA-1800 device (Automated Immunoassay Analyzer; TOSOH Bioscience, Tokyo, Japan).

Calculations and statistical analysis

All statistical analyses were done using R version 3.6.2 (R Core Team, 2019). The pig was the experimental unit. The average postprandial plasma concentrations of AA, glucose, urea, and insulin were analysed using a generalised additive mixed model (GAMM function) of the gam package of R (Hastie, 2019). The model included time of blood sampling and diet as fixed effects and the pig as the random effect. Time was declared as a repeated measure and a smoothing variable. A difference was considered to be significant at $P < 0.05$ for all statistical analyses done.

Plasma AA concentrations were further analysed using nonlinear regression with a modified compartmental Erlang function (van Milgen et al., 2022). The model assumes the existence of a target AA concentration that the animal tries to maintain. This target concentration may be affected by the ingestion of the test meal, resulting in a model with either three or four parameters (van Milgen et al., 2022). The 3-parameter model was used to describe postprandial plasma kinetics when the initial and final concentrations were similar while the 4-parameter model was used when these concentrations differed. Depending on the AA, the models used in the experiment were as follows:

$$\text{3-parameters}: \quad C_{(t)} = C_0 + (1/2AUC\lambda^3 t^2 \exp(-\lambda t))$$

$$\text{4-parameters}: \quad C_{(t)} = C_0 + (1/2AUC\lambda^3 t^2 \exp(-\lambda t)) + (C_\delta(1 - \exp(-\lambda t))$$

where $C_{(t)}$ (μM) is the plasma AA concentration at the time of sampling (t, min), C_0 (μM) the target AA concentration during fasting (i.e., at $t = 0$), C_δ (μM) the change in the target AA concentration relative to C_0, AUC (μM·min) the area under the curve over and above the target AA concentration, and λ (1/min) the rate of the postprandial change in AA concentration. For the 4-parameter model, λ is also the rate of change of the target AA concentration.

The choice of the model was done on a per AA basis to compare the values of parameters using the same model between diets. First, the individual plasma concentrations of the 20 proteinogenic AA were fitted using the 4-parameter model using the NLS2 function (nls2 package (Grothendieck, 2013)) of R. Regardless of the diet, if C_δ differed from zero for at least half the pigs (≥12 pigs) for a particular AA, the 4-parameter model was used. In the other cases, the plasma concentrations were refitted using the 3-parameter model. The effect of the diet on each parameter was tested using the LMER function using the lmerTest package (Kuznetsova et al., 2017) with diet as a fixed effect and pig as a random effect.

The apparent appearance of dietary AA in the plasma after ingestion of the meal (Q; μM) was calculated as $AUC \times \lambda$. The differences in Q between two experimental diets may be due to a difference in AA supply or to a difference in the first-pass metabolism of dietary AA by the intestine and the liver. This was assessed by computing the ratio between the Q of two treatments and the ratio between the AAs supplied by the two dietary treatments. This ratio estimates the extent to which dietary AAs are metabolised differently in the first pass between two diets. This ratio was computed for HYD over INT ($Q_{HYD:INT}/diet_{HYD:FAA}$), for FAA over INT ($Q_{FAA:INT}/diet_{FAA:INT}$), and for HYD over FAA ($Q_{HYD:FAA}/diet_{HYD:FAA}$), and expressed as percentages.

Results

General observations

Except for one pig that needed to be replaced, most of the pigs appeared healthy during the experiment. The AA profiles of the diets were similar to the expected values. Although for FAA diet, some AAs were somewhat lower than expected (i.e., Glu, Gly, Ser, and Thr).

Postprandial plasma kinetics of metabolites and insulin

The average postprandial plasma concentrations of insulin and urea are given in Fig. 1. The concentration of plasma urea after an overnight fast was higher for INT compared to the other diets. The postprandial plasma insulin concentration was highest for FAA and lowest for INT while that of urea was higher for pigs fed INT compared to pigs fed HYD or FAA. As seen in Supplementary Fig. S1, the postprandial plasma glucose concentration was higher for HYD- and FAA-fed pigs compared to INT-fed pigs.

Modelling the postprandial kinetics of plasma amino acids

The 4-parameter model was used for Arg, Cys, Gln, Glu, Ile, Leu, Val, Phe, Pro, Ser, Thr, and Tyr, which all had a positive C_δ. For the other proteinogenic AAs, the 3-parameter model was used. The fitting of the 3-parameter model is illustrated in Fig. 2A for the postprandial plasma concentrations of Met where the ingestion of the meal induced a bell-shaped curve and the initial fasting concentration was similar to the final concentration. Fig. 2B and C shows the kinetics of Val and Thr and illustrates how feeding INT resulted in a relatively flat curve, whereas feeding HYD or FAA results in an asymmetrical bell-shaped curve where the final concentration was higher than the initial concentration (i.e., $C_\delta > 0$).

Tables 2 and 3 present the parameter estimates for C_0 and C_δ, and for AUC and λ, respectively. Pigs fed INT had higher values of

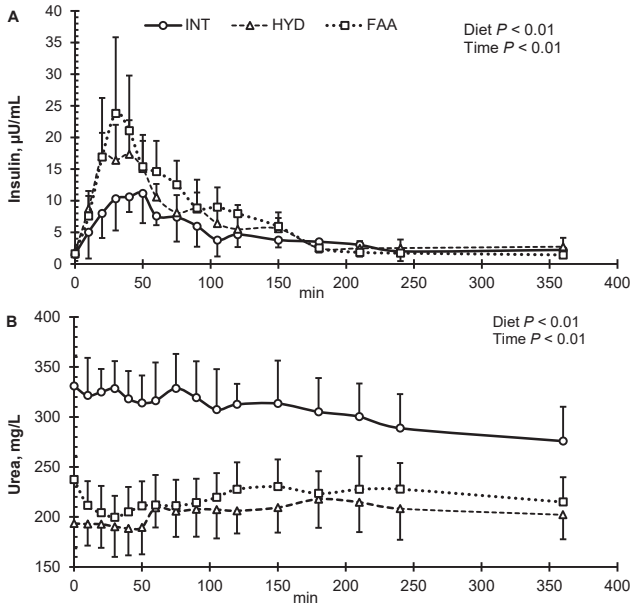

Fig. 1. Postprandial plasma kinetics (mean ± SD) of insulin (A) and urea (B) in pigs (n = 24) after feeding a diet whose amino acids originated from protein (INT), extensively hydrolysed protein (HYD), or free amino acids (FAA).

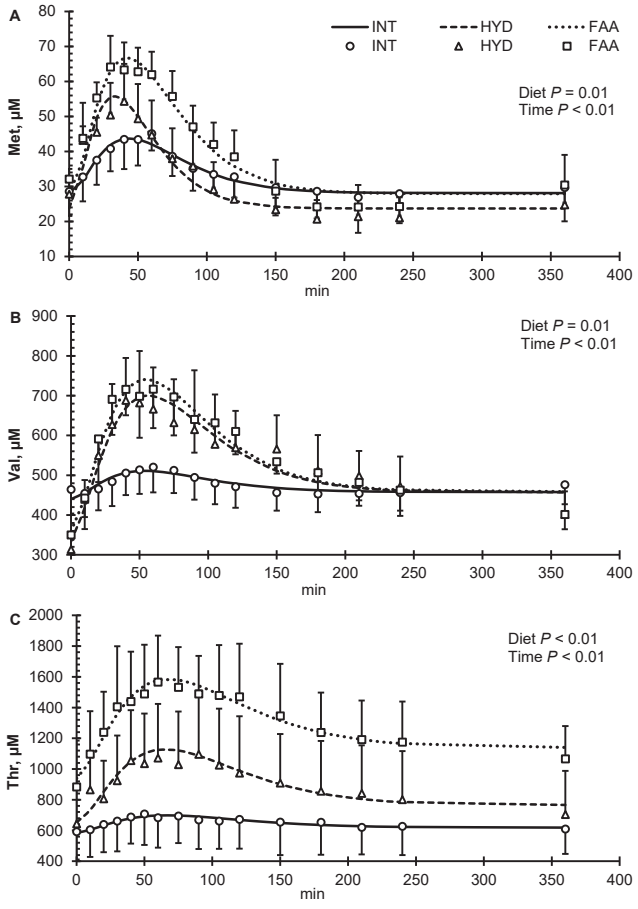

Fig. 2. Observed (symbols; mean ± SD) and estimated (lines) postprandial concentrations of plasma Met (A) using the 3-parameter model and plasma Val (B) and Thr (C) using the 4-parameter model of pigs (n = 24) after feeding a diet with amino acids originating from protein (INT), extensively hydrolysed protein (HYD), or free amino acids (FAA).

C_0 for Arg, His, Ile, and Val than the two other diets. Pigs fed HYD had the highest C_0 of Trp while those fed FAA had the highest C_0 for Cys and Thr. Pigs fed INT had generally lower estimates of C_δ, *AUC*, and λ. For C_δ, HYD had a higher estimate for Leu while FAA had the highest C_δ for Pro and Thr. The *AUC* of Trp was highest for FAA and lowest for INT. The λ of Ala, Asn, and Gln was highest for HYD and lowest for INT. The C_0 of Cys, Gly, and Thr was higher for pigs fed FAA than for those fed HYD. The *AUC* of Arg, Asn, Gly, His, Leu, Met, and Trp was higher for the pigs fed FAA, while the λ of Met was higher for pigs fed HYD. The $Q_{HYD:INT}/diet_{HYD:INT}$ and $Q_{FAA:INT}/diet_{FAA:INT}$ were all above 100% for all AAs (Fig. 3). Also, the $Q_{HYD:FAA}/diet_{HYD:FAA}$ was below 100% for all AAs, except for Cys (Fig. 4).

Discussion

The objective of this study was to assess the form of the dietary AA supply on the postprandial plasma concentrations of AA, urea, and insulin in growing pigs. To gain insight into the interpretation of the postprandial plasma AA kinetics, a model was used that described the changes in plasma concentration over time using three or four parameters with biological meaning (van Milgen et al., 2022). It was hypothesised that the appearance of AA from proteins in the plasma was gradual because of the need for proteins to be digested, while free AA and small peptides are quickly absorbed and readily available for absorption (Wu, 1998; Krehbiel and Matthews, 2003; Bos et al., 2003). To ensure that pigs ate the experimental diets, a small amount of the standard starter diet was mixed with the experimental diets and this was the same for all diets (10%). Nevertheless, 85% of the AA intake was provided by the experimental diets and most of the observed responses would be due to the experimental diets. The measured plasma concentrations of AA as well as the other metabolites and insulin were in accordance with other experiments conducted in growing pigs (Bertolo et al., 2000; Zem Fraga et al., 2021).

Despite the fact that the three diets were formulated to provide the same amount of each AA, we observed some deviations between the expected and analysed AA contents. The concentration of Cys was half in INT compared to the other two diets, which had more than double the amount of this AA. This can be explained by the processing involved in the production of feather meal that converted some Cys to lanthionine (Papadopoulos et al., 1986). The concentration of Trp was also lower for INT as feather meal is low in this AA. L-Trp was not added in diet INT as this would have meant providing it in free form, which could impact some of the measured traits, particularly the *AUC*. The *AUC* can be interpreted as the metabolic exposure of the animal induced by the appearance of dietary AA in the plasma after first-pass metabolism by the small intestine and the liver and clearance of AA due to metabolism by peripheral tissues like the skeletal muscle. Metabolism includes the incorporation of AA into proteins, AA catabolism, or their conversion to other metabolites. The lower *AUC* of Cys and Trp for INT-fed pigs than the other treatments probably resulted from a lower Cys and Trp intake.

Feeding proteins induced a modest and gradual increase in plasma AA concentrations that resulted in a low *AUC* compared to pigs fed HYD and FAA. These observations are consistent with the low digestibility of AA from feather meal (Knabe et al., 1989; Sulabo et al., 2013). Other studies found that the appearance of dietary AA in the plasma is less and slower for proteins than for free AA (Koopman et al., 2009). The parameter λ characterises the change in plasma AA concentrations, and a high value of λ results in a more rapid increase and decrease of AA plasma concentration. The λ of HYD and FAA was generally higher than that of INT. The rate of appearance and the amount of dietary AA that

Table 2
Parameter estimates describing the initial concentration and change in target concentration of the postprandial plasma amino acid kinetics of growing pigs fed a diet that supplies amino acids in different forms.[1]

Amino acid	C_0, µM					C_δ, µM				
	INT	HYD	FAA	RSD	P_{Diet}	INT	HYD	FAA	RSD	P_{Diet}
Ala	322	314	361	42.4	0.08	.	.	.	.	.
Arg	116[a]	70.1[b]	82.0[b]	21.6	<0.01	46.6	44.0	39.4	27.0	0.86
Asn	46.4	47.6	50.8	7.03	0.46	.	.	.	.	.
Asp	5.24	5.26	5.20	1.04	0.99	.	.	.	.	.
Cys	26.3[ab]	22.3[b]	33.0[a]	6.74	0.01	7.65[b]	15.9[a]	17.7[a]	7.89	0.04
Gln	392	405	401	49.5	0.87	55.8	45.8	33.3	42.7	0.57
Glu	73.9	68.9	70.3	14.3	0.81	6.06	6.60	0.75	6.5	0.18
Gly	1 103	1 138	1 405	252	0.07	.	.	.	.	.
His	59.9[a]	51.7[ab]	42.7[b]	12.4	0.04	.	.	.	.	.
Ile	118[a]	87.9[b]	98.2[ab]	16.7	<0.01	15.5	30.0	15.1	17.2	0.17
Leu	149	122	137	22.2	0.07	10.9[b]	55.2[a]	40.0[ab]	29.4	0.02
Val	440[a]	334[b]	359[b]	49.4	<0.01	17.8[b]	123[a]	99.5[a]	60.0	<0.01
Lys	55.9	48.4	45.3	22.7	0.64	.	.	.	.	.
Met	28.1	23.7	27.9	5.64	0.24	.	.	.	.	.
Phe	96.4	91.5	83.8	21.2	0.50	23.1	49.9	40.5	23.8	0.10
Pro	194	203	217	28.9	0.29	89.8[b]	117[ab]	173[a]	61.0	0.04
Ser	161	180	178	63.4	0.81	51.5[b]	128[a]	134[a]	62.2	0.03
Thr	584[b]	652[ab]	939[a]	238	0.02	34.3[b]	113[ab]	200[a]	86.3	<0.01
Trp	34.0[b]	44.0[a]	40.9[ab]	5.88	<0.01	.	.	.	.	.
Tyr	59.4	68.8	61.9	15.8	0.48	17.6[b]	50.5[a]	64.5[a]	21.4	<0.01

[1] Parameter estimates (least square means) for the target concentration after fasting (C_0) and the change in target concentration due to ingestion of the meal (C_δ) of the modified Erlang function (see text for details) from 24 pigs fed a diet with amino acids originating from protein (INT), extensively hydrolysed protein (HYD), or free amino acids (FAA).
[a,b] Values within a row with different superscript letters differ ($P < 0.05$).

Table 3
Parameter estimates describing the area under the curve and the fractional flow rate of the postprandial plasma amino acid kinetics of growing pigs fed a diet that supplies amino acids in different forms.[1]

Amino acid	*AUC*, µM·min x10^2					λ, 1/min $\times 10^{-2}$				
	INT	HYD	FAA	RSD	P_{Diet}	INT	HYD	FAA	RSD	P_{Diet}
Ala	220	285	364	112	0.06	2.53[b]	3.33[a]	2.93[ab]	0.58	0.04
Arg	61.1[b]	157[a]	227[a]	55.7	<0.01	3.21	4.56	3.82	1.29	0.14
Asn	18.1[b]	19.1[b]	31.3[a]	6.67	<0.01	3.05[b]	5.23[a]	4.31[ab]	1.39	0.02
Asp	0.63[b]	5.81[a]	4.94[a]	2.71	<0.01	9.41	3.28	4.23	4.89	0.06
Cys	13.1[b]	105[a]	35.9[ab]	67.6	0.04	5.43	2.75	4.30	2.43	0.11
Gln	95.4	107	146	94.9	0.55	2.60[b]	4.28[a]	3.77[ab]	1.23	0.04
Glu	12.3	32.5	29.8	17.3	0.08	7.46	4.04	4.62	3.36	0.15
Gly	943	772	1,306	459	0.09	0.92[b]	2.87[a]	2.19[a]	1.04	0.01
His	16.3[b]	23.4[ab]	32.2[a]	7.55	<0.01	3.15	4.57	4.12	1.17	0.07
Ile	18.4[b]	110[a]	136[a]	29.3	<0.01	4.12	5.02	4.62	0.94	0.19
Leu	28.1[b]	175[a]	233[a]	49.0	<0.01	4.51	5.09	4.48	1.06	0.45
Val	53.2[b]	254[a]	285[a]	72.5	<0.01	3.93	4.11	3.96	0.83	0.90
Lys	72.8[b]	77.3[b]	115[a]	25.8	<0.01	3.36[b]	4.85[a]	4.48[a]	0.60	<0.01
Met	12.6[b]	19.5[b]	30.2[a]	5.69	<0.01	4.65	6.65	4.78	1.70	0.05
Phe	27.4[b]	111[a]	151[a]	40.7	<0.01	3.19	4.18	3.42	1.12	0.21
Pro	112[b]	512[a]	628[a]	177	<0.01	2.24[b]	2.85[a]	2.86[a]	0.50	0.03
Ser	21.8[b]	413[a]	455[a]	210	<0.01	3.11	3.00	2.83	0.79	0.78
Thr	98.7[b]	441[a]	558[a]	167	<0.01	3.34	3.51	3.16	0.96	0.77
Trp	9.86[c]	18.8[b]	25.9[a]	4.96	<0.01	4.46	4.73	4.39	0.72	0.62
Tyr	14.8[b]	72.0[a]	86.3[a]	27.9	<0.01	3.65	3.89	3.48	0.99	0.71

[1] Parameter estimates (least square means) for the area under the curve (*AUC*) and the fractional flow rate (λ) of the modified Erlang function (see text for details) from 24 pigs fed a diet with amino acids originating from protein (INT), extensively hydrolysed protein (HYD), or free amino acids (FAA).
[a,b] Values within a row with different superscript letters differ ($P < 0.05$).

appears in the plasma after a meal can be influenced by factors like the digestibility of the protein source and the first-pass metabolism of the intestine and the liver. Since INT still needs to be hydrolysed by digestive enzymes, the postprandial appearance of its constitutive AA is slower and the changes in plasma AA concentrations are weak compared to HYD and FAA. This also means that the amount of AA that appears per unit of time is less for INT than for HYD and FAA. In this experiment, even if we cannot ignore the influence of digestibility on plasma AA concentrations, we mainly discuss how first-pass AA metabolism can be affected by the difference in the apparent quantity of AA appearing in the plasma due to a difference in dietary AA supply. The ratio of the average Q and dietary supply between two diets is indicative of the difference in first-pass AA metabolism in the intestine and liver between two diets. The observation that $Q_{HYD:INT}/diet_{HYD:INT}$ and $Q_{FAA:INT}/diet_{FAA:INT}$ was higher than 130% (Fig. 3) indicates that more AAs were used in the first pass by the intestine and liver when feeding diet INT compared to diets HYD or FAA, which agrees with results of others (Metges et al., 2000; Daenzer et al., 2001; Wu, 2009). The rapid absorption of AA from HYD and FAA seemed to be associated with a lower intestinal and hepatic metabolism in the first pass. This observation may explain the higher urea concentrations mea-

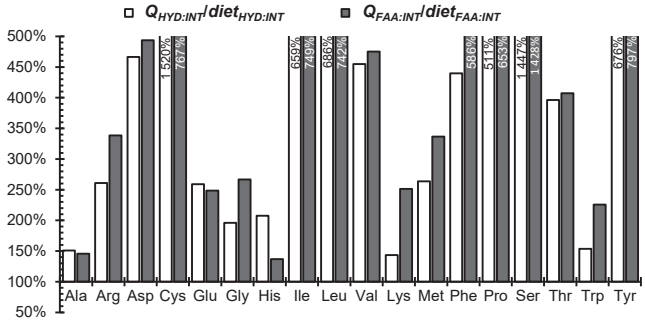

Fig. 3. The change in the apparent quantity of amino acids arriving in the plasma of pigs (n = 24) after feeding relative to the difference in the dietary amino acid supply of the intact protein diet to either the extensively hydrolysed protein diet ($Q_{HYD:INT}/diet_{HYD:INT}$) or the free amino acid diet ($Q_{FAA:INT}/diet_{FAA:INT}$).

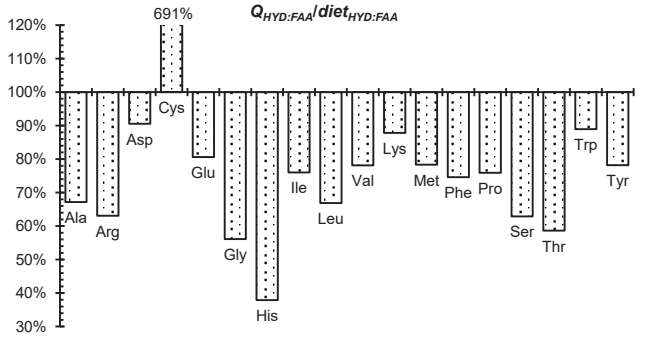

Fig. 4. The change in the apparent quantity of amino acid arriving in the plasma of pigs (n = 24) after feeding relative to the difference in the dietary amino acid supply of the extensively hydrolysed protein diet over the free amino acid diet ($Q_{HYD:FAA}/diet_{HYD:FAA}$).

sured both in the fasted and fed state in pigs fed INT compared to those fed FAA and HYD. The concentration of urea in the plasma is an indicator of AA catabolism occurring mainly in the liver (Brown and Cline, 1974). In the fed state, it reflects the catabolism of dietary AA whereas in the fasted state, it reflects the catabolism of AA released by protein breakdown. Accordingly, pigs fed proteins may have a higher protein breakdown during an overnight fast, which also explains the higher C_0 for most AAs in INT. Hence, after an overnight fast, pigs fed INT seemed to be in a more catabolic state than pigs fed FAA and HYD, similar to the findings of other studies (Kampman-van de Hoek et al., 2016).

Feeding HYD and FAA induced higher concentrations and a higher peak of plasma insulin compared to INT. Insulin is the main anabolic hormone that induces muscle protein synthesis (Davis et al., 2002; O'Connor et al., 2003). The insulin concentration only marginally increased after feeding INT. The postprandial change in AA concentration was more prominent (i.e., larger *AUC* and a higher peak concentration) for most AAs for HYD and FAA than for INT, and this may be attributed to differences in absorption rates rather than to rates of AA utilisation for protein synthesis. It is difficult to assert how the shape (i.e., flat or bell-shaped) or rate of appearance in the plasma (i.e., slow or rapid) is associated with the rate of protein synthesis. However, AAs are not only constituents of body proteins but also act as anabolic signals (Groen et al., 2015). A more marked postprandial AA concentration may therefore result in a higher signal for protein synthesis. However, this was not confirmed in broilers where no difference in the net protein synthesis after feeding proteins and free AA was observed (Chrystal et al., 2020). Otherwise, the metabolic signals of feeding proteins and free AA start not only as AAs appear in the plasma but also in the stomach and the intestine. The tertiary and quaternary structures of the protein may be detected by the intestinal tissues, which may affect the post-absorptive metabolism. However, there is limited information about this in the literature (Jahan-Mihan et al., 2011; Piumetti, 2022).

Even though feeding HYD and FAA induced very similar postprandial responses, there were some noticeable differences. An important difference between FAA and HYD is that there are still peptides in the latter. However, these are small peptides that are still readily absorbed which suggests that the differences we observed between the two diets were seen only after they are absorbed by the enterocytes. Feeding FAA generally resulted in a greater *AUC* compared to feeding HYD. This was even more apparent in the $Q_{HYD:FAA}/diet_{HYD:FAA}$ ratio, which was lower than 100% for most AAs (see Fig. 4). An explanation might be because of the small peptides present in HYD, which may have been more metabolised in the intestine and liver compared to free AA (Hara et al., 1984; Ten Have et al., 2007). It is generally believed that peptides are not transported out of the intestinal cells and they first need to be hydrolysed into their constituent AAs before being transported to the peripheral tissues (Krehbiel and Matthews, 2003).

Conclusion

In summary, providing AA in protein form or as free AA and small peptides affects the postprandial kinetics of AA, urea, insulin, and glucose. Feeding INT results in relatively flat plasma kinetics and lower plasma concentrations of AA compared to feeding HYD and FAA. This means that the form of AA in the diet of pigs has an influence in their bioavailability, but it remains unclear if the response of feeding proteins is to be interpreted positively (lower metabolic exposure and higher AA utilisation for protein synthesis) or negatively (higher urea concentrations, lower insulin concentrations). While the findings of this experiment may be true for protein sources like feather meal, they cannot be extrapolated to other protein sources with different properties.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2022.100684.

Ethics approval

The experiment followed the ARRIVE animal experimentation guidelines and the EU directive 2010/63/EU for animal experiments. The protocol was approved by the regional ethical committee, and the experiment was granted authorisation (APAFiS # 27196-2020091509061764 v2) by the Ministère de l'Enseignement Supérieur, de la Recherche et de l'Innovation.

Data and model availability statement

Experimental data that support the study findings were deposited in the official repository of Data INRAE (https://doi.org/10.57745/WHZUOQ) and are available to the public.

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Declaration of interest

The authors have no relevant interests to disclose.

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