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Nutritional quality of proteins from two beef co-products as determined in the growing pig

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Abstract

The increasing demand for food and especially proteins leads to the search for alternative protein sources. Meat co-products, which are available but little used in human food, provide a potential solution to this challenge. The present study aimed to evaluate the nutritional quality of two beef protein ingredients (greasy greaves recovered proteins (GGRP) and water recovered proteins (WRP)), both co-products of the fat rendering process. Their true ileal digestibility (TID), digestible indispensable amino acid score (DIAAS) and kinetics of plasma amino acids (AA) were measured in ten growing pigs, each fed the two co-products and a protein-free diet. Titanium dioxide was used as an indigestible marker. Digesta samples were collected for 9 h after meal ingestion, and blood samples were collected at ten time points during the same period. Total nitrogen (N) and AA contents were determined. Data were statistically analysed using linear mixed models. The TID of total N was not different between WRP and GGRP (81–84%, $P > 0.05$). The first-limiting AA was Trp for both ingredients, with a DIAAS much higher for GGRP than for WRP (74 and 10% for adults, respectively; $P < 0.001$). Postprandial plasma AA concentration peaked earlier for WRP (3 h) than for GGRP (5 h). Plasma concentrations of total and essential AA were higher ($P < 0.001$) with GGRP diet than WRP diet. Overall, GGRP has a nutritional quality suitable to meet the needs of adults for AA, while WRP needs to be supplemented with other protein sources to fulfil the dietary requirements.

Keywords: Beef co-product: Protein: Collagen: True digestibility: DIAAS

The global forecast indicate a growing demand for food and especially proteins, which leads to the search for alternative sources of proteins, while limiting the environmental impact of agriculture⁽¹⁾. In addition, the fight against hunger and malnutrition in poor countries is one of the main concerns of international agencies since the creation of the FAO of the UN-FAO/WHO Expert Committee on Nutrition⁽²⁾. Therefore, the development of protein ingredients from co-products, such as meat co-products that are little used in human food, could be a potential solution to this challenge⁽³⁾, provided that these ingredients are of good nutritional quality.

To date, the nutritional quality of proteins can only be determined with certainty by *in vivo* experiments, the growing pig model being recommended when experiments in humans are not possible, due to the proximity of its digestive physiology to that of humans⁽⁴⁾. Several studies have shown a good correlation regarding protein digestibility between humans and

pigs^(5,6). Furthermore, measuring ileal amino acid (AA) digestibility is preferable to faecal AA digestibility to assess accurately the net absorption of dietary AA by the host⁽⁷⁾, as the colonic fermentation modifies the profile of the residual dietary AA. Ileal AA digestibility, combined with the AA profile of the dietary proteins, makes it possible to determine the digestible indispensable amino acid score (DIAAS), such as recommended by FAO⁽⁷⁾ to assess the quality of proteins in foods and food ingredients for humans. Concomitantly, the plasma concentration of AA can be monitored as an indicator of the AA release kinetics, a factor that can modulate their postprandial metabolic fate towards an anabolic or catabolic pathway^(8,9).

The present work aimed to evaluate the nutritional quality of two protein ingredients from bovine co-products: greasy greaves recovered proteins (GGRP) and water recovered proteins (WRP), obtained during the beef fat rendering process and not yet characterised. The two protein ingredients are currently an

Abbreviations: AA, amino acids; CP, crude protein; DIAA, digestible indispensable amino acid; DIAAS, digestible indispensable amino acid score; EAA, essential amino acids; GGRP, greasy greaves recovered proteins; TID, true ileal digestibility; WRP, water recovered proteins.

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untapped source in human food, although they are fully compliant with the European regulations⁽¹⁰⁾. Moreover, the functional properties of these protein ingredients (gelling and emulsifying properties) indicate that they could be potential ingredients for food texturing⁽¹¹⁾. In order to facilitate their use in human food, in particular for protein enrichment for specific populations, it is first necessary to evaluate the nutritional quality of these protein ingredients through the measurement of their true ileal digestibility (TID) of total nitrogen (N) and AA, their DIAAS and the kinetics of plasma AA release as determined in the growing pig. The aim of this study was therefore to position these two protein ingredients in relation to currently available dietary proteins, on the basis of these different nutritional criteria. Because of their origin, it seemed particularly relevant to compare them with beef meat^(12,13), which offers a TID > 90% and a DIAAS close to 100.

Materials and methods

All procedures were in accordance with the European Community guidelines for the use of laboratory animals (L358-86/609/EEC). The study was approved by the local committee for ethics in animal experimentation and by the French Ministry of Higher Education and Research (agreement number: D3527532).

Animals and diets

Ten male growing pigs (Large White × Land Race × Pietrain), three-month-old and an initial average body weight (BW) of 35.6 kg (± 2.0), were used. Pigs were acclimatised for 7 d in individual pens with slatted floors. The room temperature was maintained between 21 and 24 °C (thermoneutral zone), with a 12 h light/dark cycle. One week before starting the experiment, the pigs were surgically fitted, under general anaesthesia, with a T-shaped cannula (silicone rubber) in the ileum (8 cm insert into the lumen of the digestive tract)⁽¹⁴⁾ and a catheter (polyvinyl chloride; 1.02 mm internal diameter, 2.16 mm outer diameter) in the jugular vein (about 20 cm inside the blood vessel)⁽¹⁵⁾. To avoid any risk of infection after the operation, the animals received one dose of antibiotic on the day of the operation and then five doses until the third day after the operation (Septotryl at a dose of 2 ml/30 kg BW intravenously).

Four diets were formulated for the study: a basal diet, two experimental diets containing either WRP or GGRP as protein ingredient and a protein-free diet (Table 1). During the acclimatisation and recovery period following the surgical procedure, pigs were fed a basal diet. The experimental diets were formulated in accordance with FAO recommendations, with a target of 10% dietary proteins on a DM basis^(4,16). The tested proteins were dehydrated beef proteins, GGRP and WRP, both derived from bovine co-products and supplied by a local factory (CORNILLE sas, France). The GGRP is obtained during the fat rendering process of beef. The WRP is derived from water recovery during the fat rendering process of beef and during the bone degreasing process^(11,17). The characterisation of these two protein ingredients was determined in a previous article⁽¹¹⁾. Each protein ingredient was included in one experimental diet as the

sole source of crude protein (CP), so that the diets were isonitrogenous. A protein-free diet was used to estimate basal endogenous losses of nitrogen (N) and AA. Titanium dioxide (TiO₂, 3 g/kg) was included as an indigestible solid phase marker for the calculation of the ileal digestibility of AA⁽¹⁸⁾. Vitamins and minerals were included in all diets to meet current requirement estimates for growing pigs⁽¹⁹⁾. As the WRP ingredient contained more NaCl than the GGRP ingredient (6.44% and 0.58%, respectively), an adjustment in NaCl content was required in the GGRP and protein-free diets. Thus, to obtain the same NaCl content as the WRP diet (9.43 g/kg), 8.74 g/kg NaCl was added to the GGRP diet to supplement the NaCl provided by the GGRP ingredient (0.69 g/kg); 9.43 g/kg NaCl was added to the protein-free diet.

Experimental design

The experiment took place in two blocks of five animals each (online Supplementary Fig. S1). Each pig was fed the two experimental diets (WRP and GGRP) for 4.5 d each in a crossover design. The protein-free diet was then fed after the two experimental diets, also for 4.5 d. The first block consisted of three pigs fed first the WRP diet, then the GGRP diet, and finally the protein-free diet, and two pigs fed first the GGRP diet, then the WRP diet, and finally the protein-free diet. In the opposite way, the second block consisted of two pigs receiving the WRP diet first, then the GGRP diet, and finally the protein-free diet, and three pigs receiving the GGRP diet first, then the WRP diet, and finally the protein-free diet. In order to facilitate diet consumption on the sampling day, a period of adaptation to each of the diets tested was carried out during the 3.5 d preceding the sampling day, giving an increasing proportion of the diet to be tested (diet 2) and a decreasing proportion of the diet previously tested (diet 1) as follows: 75:25; 50:50; 25:75; 0:100 (diet 1:diet 2, % w:w).

The diets were mixed with water (0.5:1, diet:water, w:w) to facilitate ingestion. The pigs had free access to water and were fed twice daily at 8 a.m. and 4 p.m., except on sampling days at 5 p.m. The daily dietary ration for each pig was 0.08 × BW^{0.75} (kg), calculated on a DM basis. Pigs were weighed at each diet change, and the daily ration of each pig was adjusted according to the BW of the pig. In total, the experimental period lasted 13.5 d for each animal, comprising, for each diet (WRP, GGRP or protein-free diet), 3.5 d of adaptation to the diet and 1 sampling day (digesta and blood sampling) over a 9-h postprandial period. Adding the 12.5 d of acclimatisation and postoperative recovery, the study therefore lasted a total of 26 d for each animal.

On each sampling day (one per diet), the pigs received the experimental diet at 8 a.m. Feed was available for 15 min, so as not to affect the kinetics of AA transfer into the plasma. Feed refusals were collected and weighed where appropriate. The sampling of ileal digesta started directly after the ingestion of the test diet and lasted for the 9 following hours. Digesta were collected continuously in pre-weighed plastic bags (Whirl-Pak, 540 ml) attached to the ileal cannula. The bags contained 1.5 ml sodium benzoate (2.3 mol/l) in order to avoid bacterial growth. The mixing of sodium benzoate in the ileal digesta was carried out manually. Full bags were replaced with empty ones when



Table 1. Ingredient composition of the diets

Ingredient (g/kg)	WRP*	GGRP*	Protein-free	Basal
Maize starch (purified)	624.6	642.76	761.57	570
Sucrose	100	100	100	100
Sunflower oil	50	50	50	50
Cellulose (purified)	30	30	30	30
Vitamin–mineral premix†	5	5	5	5
Calcium carbonate	16	16	16	16
Calcium monophosphate	25	25	25	25
Sodium chloride	0	8.74	9.43	4
WRP ingredient (6.44 % NaCl)	146.4	0	0	0
GGRP ingredient (0.58 % NaCl)	0	119.5	0	0
Micellar casein isolate	0	0	0	200
TiO ₂	3	3	3	0

WRP, water recovered proteins; GGRP, greasy greaves recovered proteins; TiO₂, titanium dioxide.

* Diets were formulated to contain approximately 10 % crude protein on an as-fed basis

† The vitamin–micromineral premix provided the following quantities of vitamins and minerals per kg of complete diet: Vit. A: 1 000 000 U.I.; Vit. D₃: 200 000 U.I.; Vit. E: 4000 mg; Vit. K₃ (MNB): 870 mg; Vit. B₁-Thiamin: 400 mg; Vit. B₂-Riboflavin: 800 mg; Vit. PP-Niacin: 3000 mg; Vit. B₅-Pantothenate Ca: 2000 mg; Vit. B₆-Pyridoxine: 200 mg; Vit. H-B₈-Biotin: 40 mg; Vit. B₉-Folic acid: 200 mg; Vit. B₁₂-Cyanocobalamin: 4 mg; Choline chloride: 133 333 mg; Fe (sulfate monohydrate): 16 000 mg; Cu (sulfate pentahydrate): 2000 mg; Zn (oxide): 14 000 mg; Mn (oxide): 8000 mg; Se (selenite): 30 mg; I (calcium): 40 mg.

necessary (at least after the 1st hour, then every 2 h). Content was immediately transferred to a plastic box (previously weighed) and stored at -20°C before being freeze-dried. The freeze-dried samples were then grouped into five pools corresponding to the collection periods between 0 and 1 h postprandial, 1–3 h, 3–5 h, 5–7 h and 7–9 h. Finally, an overall sample, representative of the entire postprandial period, was prepared by mixing these five pools in proportion to the quantity of DM in each of them. On the same day as the digesta sampling, blood samples (2.5 ml per time point) were collected in heparin-lithium tubes at 30 min before and 20, 40, 60, 90, 120, 180, 300, 420 and 540 min after the diet ingestion. Plasma was immediately separated by centrifugation at 4°C for 10 min at 2500 g and stored at -80°C until AA analysis was performed.

Chemical analysis

Ingredients WRP and GGRP were analysed in triplicate for DM, total AA and total N. Diets were analysed in triplicate and ileal digesta in duplicate for DM, total AA, total N and TiO₂. Plasma samples were analysed in single for free AA content, urea and insulin.

The DM content was determined gravimetrically at $102 \pm 2^{\circ}\text{C}$ for 5 h.

The total AA content was determined according to Davies and Thomas⁽²⁰⁾. Samples were hydrolysed for 24 h in 6 mol/l hydrochloric acid at 110°C in N-sealed glass tubes. For Cys and Met, performic acid oxidation was carried out before acid hydrolysis. The AA were separated by ion exchange chromatography (Biochrom30+ automatic AA Analyzer) using a lithium citrate buffer (2.5 % final) as eluent⁽²¹⁾ and quantified photometrically after ninhydrin derivatisation (EZ NIN Kit, Biochrom). Absorbance was measured at 570 nm, except for Pro and HyPro (hydroxyproline) detection (440 nm), and AA were quantified using an external calibration curve previously established with standards (A6407 and A6282, Sigma). The Trp was analysed after

sample hydrolysis with Ba(OH)₂ for 16 h at 110°C in Teflon tubes with screw cap⁽²²⁾. The Trp was determined using an RP-HPLC system (Waters™ e2695 Separations Modul) and quantified by fluorimetry (excitation at 280 nm and emission at 346 nm). The L-Trp standard (0–10 mg/l), corrected by the internal 5-methyl-Trp standard, was used for Trp quantification.

The concentration of free AA in plasma was determined after deproteinisation of the samples⁽²³⁾, using the same ion exchange chromatography, the same ninhydrin derivatisation and the same absorbance measurement as used for total AA. For deproteinisation, plasma samples were incubated with sulfosalicylic acid solution (5 % SSA, including N-Leu internal standard) at 0°C for 1 h and then centrifuged (10 000 g, 10 min) before filtration on a $0.45\ \mu\text{m}$ membrane. Free AA were quantified using an external calibration curve previously established with standards (A6407, A6282, Gln, urea, 2.5% SSA and N-Leu, Sigma) in lithium citrate buffer. Urea content was determined concomitantly with the free AA analysis. Insulin concentration in plasma was measured using a RIA kit (Insulin-CT, CisBio Bioassays) and an AIA-1800 device (Automated Immunoassay Analyzer; TOSOH Bioscience)⁽²⁴⁾.

Total N in diet and digesta samples was analysed by the Dumas method on a LECO FP 828 analyser after calibration using EDTA. The N to protein conversion factor of 6.25 was used to determine their CP content⁽²⁵⁾.

The TiO₂ content was determined based on a method adapted from Mudunkotuwa *et al.*⁽²⁶⁾. Mineralisation by microwave-assisted acid digestion was first carried out (ETHOS UP, Thermo Fisher Scientific). For this, 0.25 g of samples were heated to 210°C in the presence of acid (2 ml HNO₃ 98 % + 4 ml H₂SO₄ 69 %) in Teflon digestion vessels, with a ramp time of 25 min and a holding time of 15 min. Then 30 ml of ultrapure H₂O were added after sample cooling. The TiO₂ content was then measured using inductively coupled optical emission spectrometry (ICP-OES, Thermo Fisher Scientific) using an external calibration curve (0–10 mg/l TiO₂). All samples were diluted in HNO₃ 5 % before analysis.

Data analysis

Total N flow was calculated, according to Hodgkinson *et al.*⁽¹⁶⁾, as follows (in mg/g dry matter intake, DMI):

$$\text{Total N flow} = [\text{Total N}]_{\text{digesta}} \times \frac{[\text{TiO}_2]_{\text{diet}}}{[\text{TiO}_2]_{\text{digesta}}} \quad (1)$$

where $[\text{Total N}]_{\text{digesta}}$ and $[\text{TiO}_2]_{\text{digesta}}$ were expressed in mg/g of DM of digesta and $[\text{TiO}_2]_{\text{diet}}$ expressed in mg/g of DM of diet.

The AA flow was calculated in the same way, replacing $[\text{Total N}]_{\text{digesta}}$ with $[\text{AA}]_{\text{digesta}}$ in Eq. 1.

The TID of N was calculated in the pooled digesta, according to Hodgkinson *et al.*⁽¹⁶⁾, as follows (in %):

$$\text{TID of N (\%)} = \frac{[\text{Total N}]_{\text{diet}} - (\text{Total N flow} - \text{Endogenous Total N flow})}{[\text{Total N}]_{\text{diet}}} \times 100 \quad (2)$$

where variables were expressed in mg/g DMI.

The TID of AA was calculated in the same way, replacing [Total N]_{diet} with [AA]_{diet}, 'Total N flow' with 'AA flow' and 'Endogenous Total N flow' with 'Endogenous AA flow', in Eq. 2.

Digestible indispensable amino acid (DIAA) reference ratio was calculated for each essential AA (EAA), according to Hodgkinson *et al.*⁽¹⁶⁾, using the AA scoring pattern of the reference protein for three different populations, namely undernourished children (0.5–5 years old)⁽²⁷⁾, healthy children (0.5–3 years old), and >3 years old children, adolescents and adults⁽⁷⁾:

$$\text{DIAA reference ratio (\%)} = 100 \times \frac{\text{mg of the EAA in 1 g of the dietary protein} \times \text{TID of the EAA}}{\text{mg of the EAA in 1 g of the reference protein}} \quad (3)$$

where 1 g of the dietary protein corresponded to 1 g of CP (total N × 6.25).

DIAAS was defined as the lowest value of DIAA reference ratio⁽⁷⁾.

Semi-dynamic digestion and analysis of digesta

The *in vitro* semi-dynamic model (INFOGEST) was used to simulate gastric digestion of both WRP and GGRP diets. The semi-dynamic digestions were carried out according to a method adapted from Mulet-Cabero *et al.*⁽²⁸⁾ using the pH-stat device with a pH probe (Porotrode, Ref 60235200, 842 Titrand, Metrohm) and tiamo™ software (Metrohm). The model included a progressive five-step addition of gastric secretions (HCl, pepsin and simulated gastric fluid), according to Musse *et al.*⁽²⁹⁾. The progressive gastric emptying was not considered in the present experiment. In this study, only the oral and gastric phases of digestion were performed, and for the oral phase only a dilution of the diets was carried out with simulated salivary fluid without enzyme. Samples (5 ml) were collected at 40, 80 and 120 min of the gastric phase to evaluate their microstructure based on confocal microscopy and particle size distribution as well as their viscosity.

Microstructures of the reconstituted diets in water (0.5:1, diet: water, w:w) and gastric digesta were observed using a laser scanning confocal microscope (ZEISS LSM880, Carl Zeiss AG) at 20× magnification, at 37 °C, as described by Chauvet *et al.*⁽³⁰⁾. The particle size distribution of these samples was determined using laser light scattering with a Malvern Mastersizer 2000 (Malvern Instrument Ltd). The refractive index of protein was set at 1.52 and that of water at 1.33. The particle diameter was expressed as the median diameter $d_{0.5}$ and the volume-weighted average diameter $d_{4.3}$ as follows:

$$d_{4.3} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (4)$$

with n_i the number of droplets of diameter d_i .

The rheology of the reconstituted diets in water (0.5:1, diet: water, w:w) and of the gastric digesta was measured using a rheometer (AR2000, EX TA Instrument) at 37 °C, as described by Wu *et al.*⁽³¹⁾ with a slight modification. The scanning range of the

steady shear rate was 0.1–500/s. The viscosity value considered for result analysis was that measured at 10/s.

Statistical analyses

Statistical analyses were performed using the R software (version 3.6.2)⁽³²⁾.

A linear mixed model (lmerTest package) was used to test the 'diet' effect and 'block' effect on the TID of total N and of each AA, with pig as a random factor. If there was no statistically significant 'block' effect, it was removed from the model.

The plasma AA, urea and insulin concentrations were statistically analysed by a linear mixed model (lmerTest package) considering the factors 'diet' (WRP and GGRP), 'time' (–30, 20, 40, 60, 90, 120, 180, 300, 420 and 540) and their interaction, with pig as a random factor. When the interaction was statistically significant, a *post hoc* test was performed to establish the pairwise comparisons (Emmeans, Tukey adjustment).

Before carrying out the analyses, normality (Shapiro–Wilk test) and homoscedasticity (Levene's tests) of the model residuals were verified ($P > 0.05$). The covariance was unstructured. Statistical significance was considered at $P < 0.05$.

Data are presented as mean ± SEM, unless otherwise mentioned.

Results

The pigs stayed healthy throughout the experimental period. Minimal leakage occurred from the ileal cannula during digesta collection, with the exception of one WRP-fed pig for which digesta sampling could not be carried out momentarily due to signs of pain, pending healing of the cannula area. They weighed 35.6 ± 2.0 kg (mean ± SD) at the beginning of the study (before surgery) and 41.6 ± 3.8 kg at the end of the study. All pigs readily consumed the diets with the exception of three pigs fed the WRP diet (diet refusal > 50%) that were thus excluded from the data analysis for both the digestibility and plasma AA dataset. Therefore, the final dataset included digestibility data for six WRP-fed pigs and ten GGRP-fed pigs, and plasma AA data for seven WRP-fed pigs and ten GGRP-fed pigs.

Crude protein and amino acid composition of the diets

The CP content of the WRP and GGRP diets, 11.4% and 12.1% on a DM basis respectively (Table 2), was slightly higher than the FAO recommendation for the measurement of protein digestibility (10% dietary protein) but in the same range between diets. Individual AA contents varied between the two diets (Table 2), with the WRP diet containing less EAA than the GGRP diet (2.47 *v.* 4.00 g/100 g, respectively). The Trp was the least abundant EAA in WRP and GGRP diets, while the most abundant was Lys in WRP diet and Leu in GGRP diet.

True ileal digestibility of nitrogen and amino acids

The TID of total N did not differ between diets despite being numerically lower for GGRP than for WRP (81.2 and 84.2%, respectively; $P > 0.05$, Table 3). Similarly, the TID of individual AA did not differ between diets, except for Ala, Arg and Glx, for



Table 2. Protein and AA composition of the experimental diets and of the ingredients containing water recovered proteins (WRP) or greasy greaves recovered proteins (GGRP)

	<i>Experimental diets*</i>				<i>Ingredients</i>			
	WRP		GGRP		WRP		GGRP	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM (g/100 g)	91.4	0.1	91.1	0.2	95.78	0.05	94.53	0.26
CP (N × 6.25) (g/100 g DM)	11.4	0.1	12.1	0.1	71.16	0.13	88.58	0.03
Essential AA	g/100 g of diet				g/100 g of ingredient			
Cys	0.04	0.00	0.11	0.01	0.27	0.01	0.83	0.06
His	0.32	0.01	0.20	0.01	1.99	0.03	1.44	0.06
Ile	0.19	0.00	0.37	0.01	1.21	0.02	2.67	0.07
Leu	0.44	0.01	0.76	0.01	2.72	0.04	5.55	0.09
Lys	0.48	0.01	0.67	0.03	2.99	0.04	4.90	0.20
Met	0.08	0.00	0.20	0.01	0.50	0.03	1.49	0.06
Phe	0.23	0.00	0.39	0.01	1.43	0.01	2.87	0.06
Tyr	0.11	0.00	0.24	0.01	0.68	0.01	1.77	0.04
Thr	0.25	0.00	0.39	0.01	1.55	0.03	2.85	0.08
Trp	0.02	0.00	0.08	0.00	0.09	0.00	0.56	0.01
Val	0.31	0.00	0.59	0.01	1.95	0.03	4.30	0.10
<i>Total</i>	<i>2.47</i>	<i>0.04</i>	<i>4.00</i>	<i>0.11</i>	<i>15.39</i>	<i>0.25</i>	<i>29.23</i>	<i>0.83</i>
Non-essential AA	g/100 g of diet				g/100 g of ingredient			
Ala	0.95	0.01	0.98	0.01	5.92	0.09	7.13	0.09
Arg	0.78	0.01	0.77	0.03	4.84	0.07	5.66	0.20
Asx	0.69	0.01	0.86	0.03	4.28	0.06	6.29	0.20
Glx	1.38	0.02	1.35	0.05	8.57	0.14	9.85	0.35
Gly	2.16	0.03	1.73	0.06	13.45	0.20	12.67	0.38
Pro	1.12	0.02	0.99	0.03	6.97	0.11	7.23	0.20
HyPro	1.00	0.02	0.69	0.03	6.25	0.10	5.05	0.24
Ser	0.35	0.01	0.42	0.01	2.18	0.03	3.08	0.08
<i>Total</i>	<i>8.43</i>	<i>0.13</i>	<i>7.79</i>	<i>0.24</i>	<i>52.46</i>	<i>0.81</i>	<i>56.96</i>	<i>1.75</i>
Total AA	10.90	0.17	11.79	0.35	67.85	1.76	86.19	2.92

CP, crude protein; AA, amino acids; Asx, Asn + Asp; Glx, Gln + Glu.

Data are presented as mean ± SD (*n* 3).

* The AA composition of the WRP or GGRP diets has been calculated based on the AA composition of the WRP or GGRP ingredients, respectively. These calculated values were used for the evaluation of AA digestibility.

which TID was higher in WRP-fed pigs ($P=0.013$, 0.009 and 0.017 for Ala, Arg and Glx, respectively).

Digestible indispensable amino acid reference ratio and digestible indispensable amino acid score

The first-limiting EAA was Trp for the WRP and GGRP diets and all the three target populations, associated with very low DIAAS values (7–10%) for the WRP (Table 4). In contrast, the DIAAS was higher for the GGRP diet ($P<0.001$), with values remaining low for young children, undernourished or healthy (49% and 57%, respectively), but reaching 74% for older children (> 3 years), adolescents and adults.

It should be noted that WRP is rich in His, with a content being 3–54% higher than that recommended for the three target populations, while on the contrary His was the second-limiting EAA in GGRP. The GGRP is rich in aromatic amino acids (AAA = Phe + Tyr), Thr and Val, but to a limited extent with contents being only 3–9% higher than the recommended value for the >3 years old children, adolescents and adults.

Postprandial kinetics of plasma amino acids, urea and insulin

The evolution of plasma AA concentration during the 9-h postprandial period is shown in Figs. 1–3. The diet and the time × diet interaction had an effect ($P<0.001$ and $=0.001$,

respectively) on the plasma concentration of total AA, with a higher concentration for the GGRP diet in the preprandial phase ($t=0$ h; $P=0.009$) and then during the first 1.5 h (Fig. 1(a)). Plasma total AA concentration peaked 3 h and 5 h after ingestion of the WRP and GGRP diets, respectively. The concentration of non-essential AA in plasma (Fig. 1(b)) followed the same patterns as that of total AA, with a similar peak at 3 h and 5 h for the WRP and GGRP diets, respectively, but without diet effect ($P>0.05$). The concentration of EAA in plasma (Fig. 1(c)) peaked 5 h after feeding for the both diets, with a diet effect ($P<0.001$). It should be noted that the plasma concentration of EAA is higher with the GGRP diet than with the WRP diet throughout the 9-h postprandial period. The EAA concentration was mainly influenced by that of Val and Leu (Fig. 2), which presented higher concentrations with the GGRP diet ($P<0.001$). Other individual AA, such as Cys, Ile, Met, Phe, Tyr and Gly, also had a higher plasma concentration with the GGRP diet ($P<0.001$). On the contrary, Pro, HyPro and Ser (Fig. 3) presented lower plasma concentrations with the GGRP diet ($P=0.01$, <0.001 and <0.001 , respectively). An effect of the time × diet interaction was also observed for Tyr and Ser ($P=0.07$ and <0.001 , respectively). Solely His, Lys, Thr, Ala, Asx (Asp+Asn) and Glx (Glu+Gln) plasma concentrations did not present any difference between diets along the entire postprandial period ($P>0.05$).

An effect of the diet was observed on plasma urea concentration, which was lower in GGRP-fed pigs than in

Table 3. True ileal digestibility of N and AA in growing pigs fed either water recovered proteins (WRP) or greasy greaves recovered proteins (GGRP) diet*†

Items	WRP		GGRP		Diet effect (<i>P</i>)
	Mean	SD	Mean	SD	
Total N (%)	84.2	3.19	81.2	2.20	0.48
Essential AA (%)					
Cys	76.9	7.11	77.5	2.64	0.91
His	87.8	2.64	82.5	1.59	0.09
Ile	88.5	4.10	85.0	1.68	0.37
Leu	89.4	3.33	85.7	1.40	0.25
Lys	88.7	2.60	83.8	1.18	0.07
Met	86.7	7.35	87.4	2.32	0.91
Phe	90.7	3.12	87.2	1.56	0.28
Tyr	82.6	5.58	83.1	1.58	0.93
Thr	84.5	4.25	83.6	1.52	0.81
Trp	49.5	15.07	77.1	3.08	0.17
Val	88.3	4.34	84.7	1.83	0.38
Mean	83.6	5.28	83.4	1.85	0.98
Non-essential AA (%)					
Ala	91.9	2.53	83.6	1.65	0.013
Arg	96.0	1.24	92.0	0.71	0.009
Asx	66.4	7.44	62.5	3.19	0.59
Glx	89.9	2.64	81.7	1.73	0.017
Gly	91.1	3.01	83.6	3.18	0.14
Pro	97.3	5.27	87.0	3.05	0.09
HyPro	86.1	4.25	79.9	3.62	0.30
Ser	87.9	3.77	83.2	1.57	0.20
Mean	88.3	3.77	81.7	2.34	0.09

AA, amino acids; Asx, Asp + Asn; Glx, Glu + Gln.

* Data are least squares means (*n* 8 for pigs fed WRP; *n* 10 for pigs fed GGRP). As no significant 'block' effect was found, only the 'diet' effect is presented. Means between the diets were compared using a linear mixed model (*lmerTest* package). A *P* < 0.05 was considered as significant (in bold).

† True ileal digestibility values were calculated by subtracting ileal endogenous losses from apparent ileal digestibility values (online Supplementary Table S1). Endogenous loss of total N was 2.42 ± 0.19 g/kg of DM intake; endogenous losses of AA (in g/kg DM intake) were as follows: Cys, 0.27 ± 0.02; His, 0.20 ± 0.01; Ile, 0.42 ± 0.03; Leu, 0.71 ± 0.05; Lys, 0.50 ± 0.04; Met, 0.23 ± 0.02; Phe, 0.42 ± 0.03; Tyr, 0.27 ± 0.03; Thr, 0.73 ± 0.05; Trp, 0.20 ± 0.02; Val, 0.58 ± 0.04; Ala, 0.60 ± 0.05; Arg, 0.45 ± 0.05; Asx, 0.97 ± 0.06; Glx, 1.24 ± 0.09; Gly, 1.34 ± 0.19; Pro, 1.89 ± 0.71; HyPro, 0 ± 0.00; Ser, 0.57 ± 0.04.

WRP-fed pigs throughout the entire postprandial period (*P* < 0.001, Fig. 4(a)). The maximum concentration of urea was observed 7 h after feeding the two diets.

The postprandial plasma concentration of insulin did not differ between diets (*P* > 0.05) and was at its maximum 20 min after feeding (Fig. 4(b)).

Structural change of the diets during *in vitro* digestion

The changes of the microstructure and viscosity of the WRP and GGRP diets during *in vitro* digestion are illustrated in Fig. 5 and Table 5.

Confocal microscopy images show protein aggregation throughout the digestion for both WRP and GGRP diets (Fig. 5(b) and (d)). This is confirmed by the increase in $d_{0.5}$ by a factor of 3 for WRP and 6 for GGRP after 120 min of digestion. This reflects the increase in peak intensity of 1000 µm-diameter particles and the shift of the peak of smaller particles (about 80 µm) to higher values (about 100 µm). However, at all times during digestion, the median particle size was lower (*P* < 0.001) in WRP digesta (from 66 to 202 µm) than in GGRP digesta (from

Table 4. Digestible indispensable amino acid (DIAA) reference ratio and DIAAS of water recovered proteins (WRP) and greasy greaves recovered proteins (GGRP) calculated on the basis of the CP content

Items	WRP	GGRP
DIAA reference ratio (%) – undernourished child (0.5–5 years old)		
His	103	56
Ile	44	75
Leu	49	77
Lys	57	71
SAA (Cys + Met)	29	71
AAA (Phe + Tyr)	42	71
Thr	51	75
Trp	7	49
Val	53	89
DIAAS (%)‡		
Undernourished child (0.5–5 years old)	7 (Trp)	49 (Trp)
DIAA reference ratio (%) – healthy child (0.5–3 years old)		
His	123	67
Ile	47	80
Leu	52	81
Lys	65	81
SAA (Cys + Met)	33	81
AAA (Phe + Tyr)	50	86
Thr	59	87
Trp	8	57
Val	56	96
DIAAS (%)‡		
Healthy child (0.5–3 years old)	8 (Trp)	57 (Trp)
DIAA reference ratio (%) – older child (>3 years), adolescent, adult		
His	154	84
Ile	50	85
Leu	56	88
Lys	78	97
SAA (Cys + Met)	39	96
AAA (Phe + Tyr)	64	109
Thr	74	108
Trp	10	74
Val	60	103
DIAAS (%)§		
Older child (>3 years), adolescent, adult	10 (Trp)	74 (Trp)

CP, crude protein; SAA, sulphur amino acids; AAA, aromatic amino acids; DIAAS, digestible indispensable amino acid score (with first-limiting AA in brackets).

The data are presented as the mean (*n* 8 for pigs fed WRP; *n* 10 for pigs fed GGRP). † DIAAS were calculated using the recommended AA scoring patterns for undernourished child (0.5–5 years old) as mg AA/g protein: His, 24; Ile, 34; Leu, 70; Lys, 65; SAA, 31; AAA, 63; Thr, 36; Trp, 10; Val, 46⁽²⁷⁾.

‡ DIAAS were calculated using the recommended AA scoring patterns for healthy children (0.5–3 years old) as mg AA/g protein: His, 20; Ile, 32; Leu, 66; Lys, 57; SAA, 27; AAA, 52; Thr, 31; Trp, 8.5; Val, 43⁽⁷⁾.

§ DIAAS were calculated using the recommended AA scoring patterns for older children (>3 years), adolescents and adults as mg AA/g protein: His, 16; Ile, 30; Leu, 61; Lys, 48; SAA, 23; AAA, 41; Thr, 25; Trp, 6.6; Val, 40⁽⁷⁾.

86 to 518 µm). At the same time, the viscosity of both diets sharply decreased along the gastric digestion by an equivalent factor (about 34), from 1.31 to 0.04 Pa.s at 10/s for WRP diet, and from 35.79 to 1.03 Pa.s for GGRP diet. At all times during digestion, WRP was at least twenty-five times less viscous than GGRP (*P* < 0.001).

Discussion

Diet composition

As previously reported⁽¹¹⁾, the difference in AA composition between WRP and GGRP diets can be explained by the differences between the two ingredients in terms of production

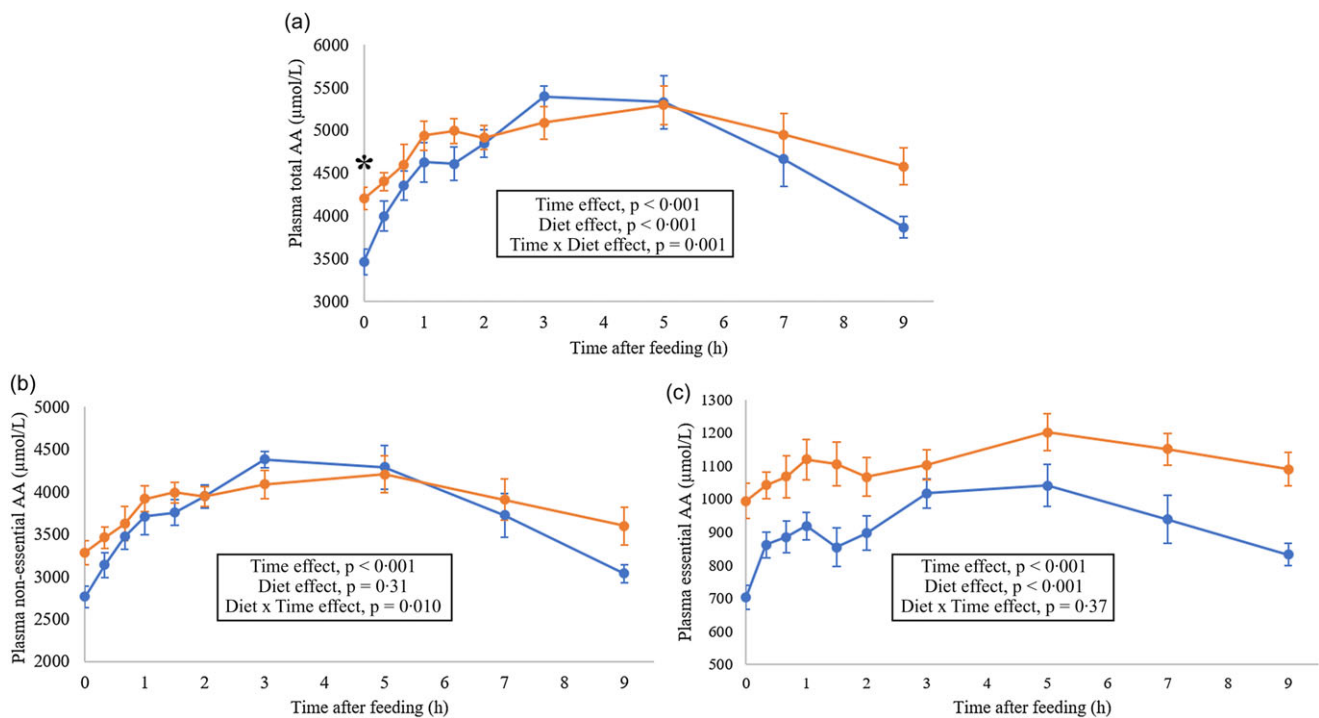


Fig. 1. Total AA concentration (a), non-essential AA concentration (b) and essential AA concentration (c) in plasma of pigs fed experimental diets containing water recovered proteins (WRP, blue, *n* 7) or greasy greaves recovered proteins (GGRP, orange, *n* 10) as the only dietary protein source. The data are presented as the mean ± SEM. Essential AA are the sum of His, Ile, Leu, Lys, Met, Phe, Thr, Val and conditionally EAA (Tyr and Cys). Non-essential AA are the sum of Ala, Arg, Asn, Asp, Gln, Glu, Gly, Pro, HyPro and Ser. The plasma AA concentrations were statistically analysed by a linear mixed model (lmerTest package) considering the factors ‘diet’, ‘time’ and their interaction, with pig as a random factor. When the interaction was statistically significant, a post hoc test was performed to establish the pairwise comparisons (Emmeans, Tuckey adjustment). A *P* < 0.05 was considered as significant and marked with an asterisk (curves). AA, amino acids.

process and the origin of the raw material, that is, different parts of the animal. The composition is close to that of bovine muscle hydrolysate for GGRP and bovine collagen hydrolysate for WRP⁽³³⁾. However, both ingredients are difficult to compare with other meat co-products described in literature, as the composition depends on each particular type of co-product and on the animal species from which they originate⁽³⁴⁾.

The greater rate of refusal observed for WRP diet may be due to the lower palatability of this ingredient. Its low content of Trp (0.02 %) could also play a role, as it has been shown that a diet deficient in this AA induces a drop in appetite⁽³⁵⁾. However, such an effect was reported after 21 d on a deficient diet, whereas here each animal consumed the WRP-based diet for 3.5 d adaptation only.

Water recovered proteins and greasy greaves recovered proteins are moderately digestible

Quantification of total AA and total N in the pool of ileal digesta collected throughout the 9-h postprandial period was used to calculate the protein digestibility of WRP and GGRP proteins, after correction for the endogenous N and AA flows determined after ingestion of a protein-free diet. Protein digestibility measured in this way is called as standardised ileal digestibility in animal nutrition⁽³⁶⁾, which corresponds to the TID in human nutrition, as reported by the FAO⁽⁴⁾.

The ileal endogenous N and total AA flows measured (2.4 and 11.6 g/kg DMI, respectively; Table 3) were consistent with the literature data⁽³⁷⁾. The TID of N did not differ between diets (*P* > 0.05) due to a high variability of the data, particularly for WRP. The values of TID were 84.2 % for WRP and 81.2 % for GGRP, which corresponds to a moderate level of digestibility, and lower than the TID value reported for bovine meat proteins (> 90 %) ^(13,38,39). This is likely due to the high collagen content of both ingredients (40.03 % ± 1.69 in WRP and 36.83 % ± 0.18 in GGRP)⁽¹¹⁾, as given that connective tissue proteins are known to be more resistant to enzymatic proteolysis⁽⁴⁰⁾. As a matter of fact, TID values measured for GGRP and WRP were similar to those reported for beef muscle hydrolysate and collagen, that is, 84 % and 79–81 %, respectively^(6,33). Although the protein digestibility of WRP and GGRP is lower than that of many animal proteins, it is noticeable that it is higher than that of most plant proteins, the use of which is encouraged for health and environmental reasons, such as maize (70–76 % TID), barley (74 % TID), sorghum (66 % TID) and even beans (41–72 % TID)^(41,42).

In the present study, WRP and GGRP ingredients were included in the diets as is, with no further process. Processing, in particular cooking, could modify protein digestibility, but in directions that can be opposite depending on the process parameters and/or protein nature. For example, cooking beef at high temperatures for a long time can moderately reduce protein digestibility, compared with cooking at a lower temperature for a



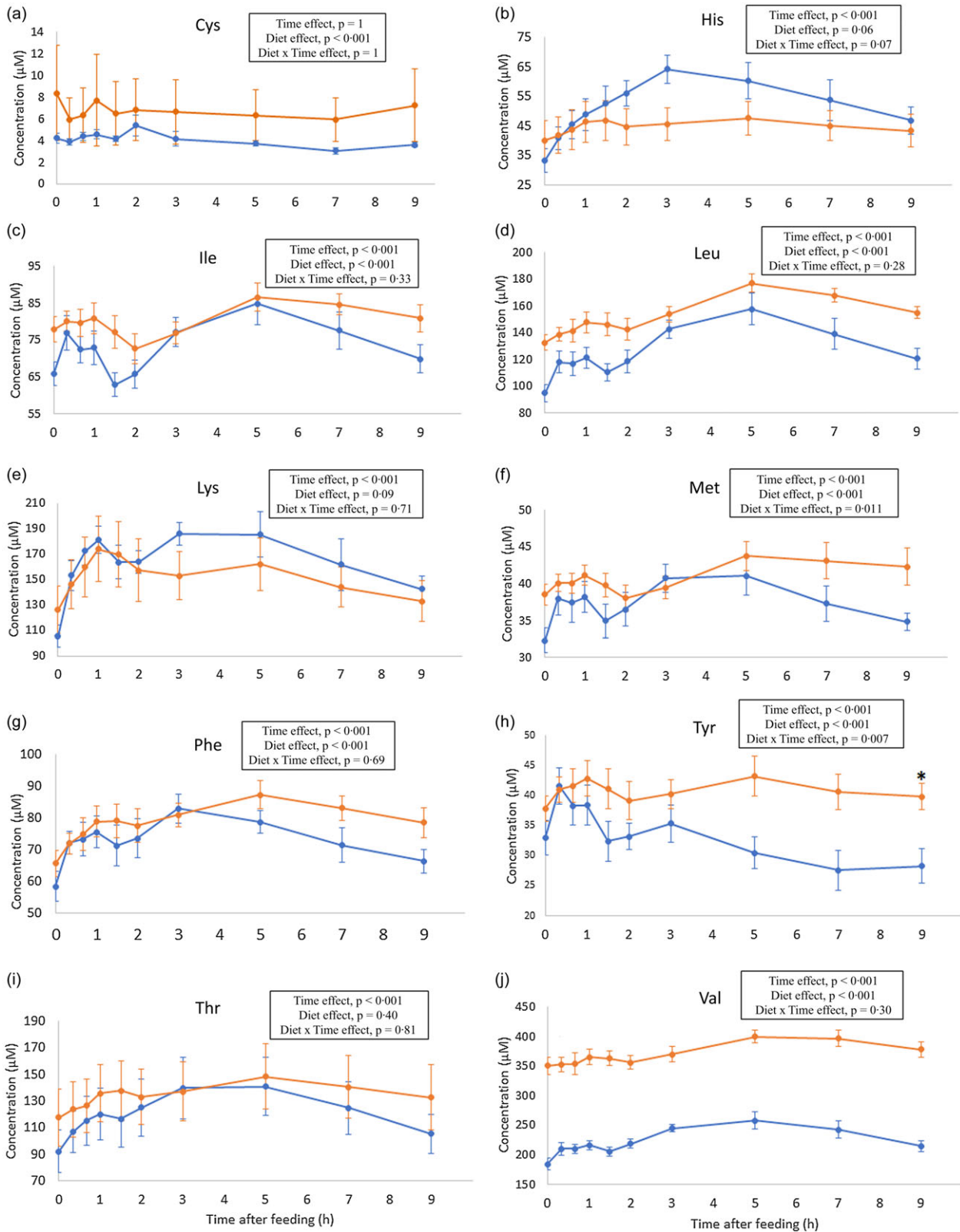


Fig. 2. Essential AA concentrations in plasma of pigs fed experimental diets containing water recovered proteins (WRP, blue, n 7) or greasy greaves recovered proteins (GGRP, orange, n 10) as the only dietary protein source. The data are presented as the mean \pm SEM. Essential AA are composed of Cys (a), His(b), Ile (c), Leu (d), Lys (e), Met (f), Phe (g), Tyr (h), Thr (i) and Val (j). The plasma AA concentrations were statistically analysed by a linear mixed model (lmerTest package) considering the factors 'diet', 'time' and their interaction, with pig as a random factor. When the interaction was statistically significant, a post hoc test was performed to establish the pairwise comparisons (Emmeans, Tuckey adjustment). A $P < 0.05$ was considered as significant and marked with an asterisk (curves).

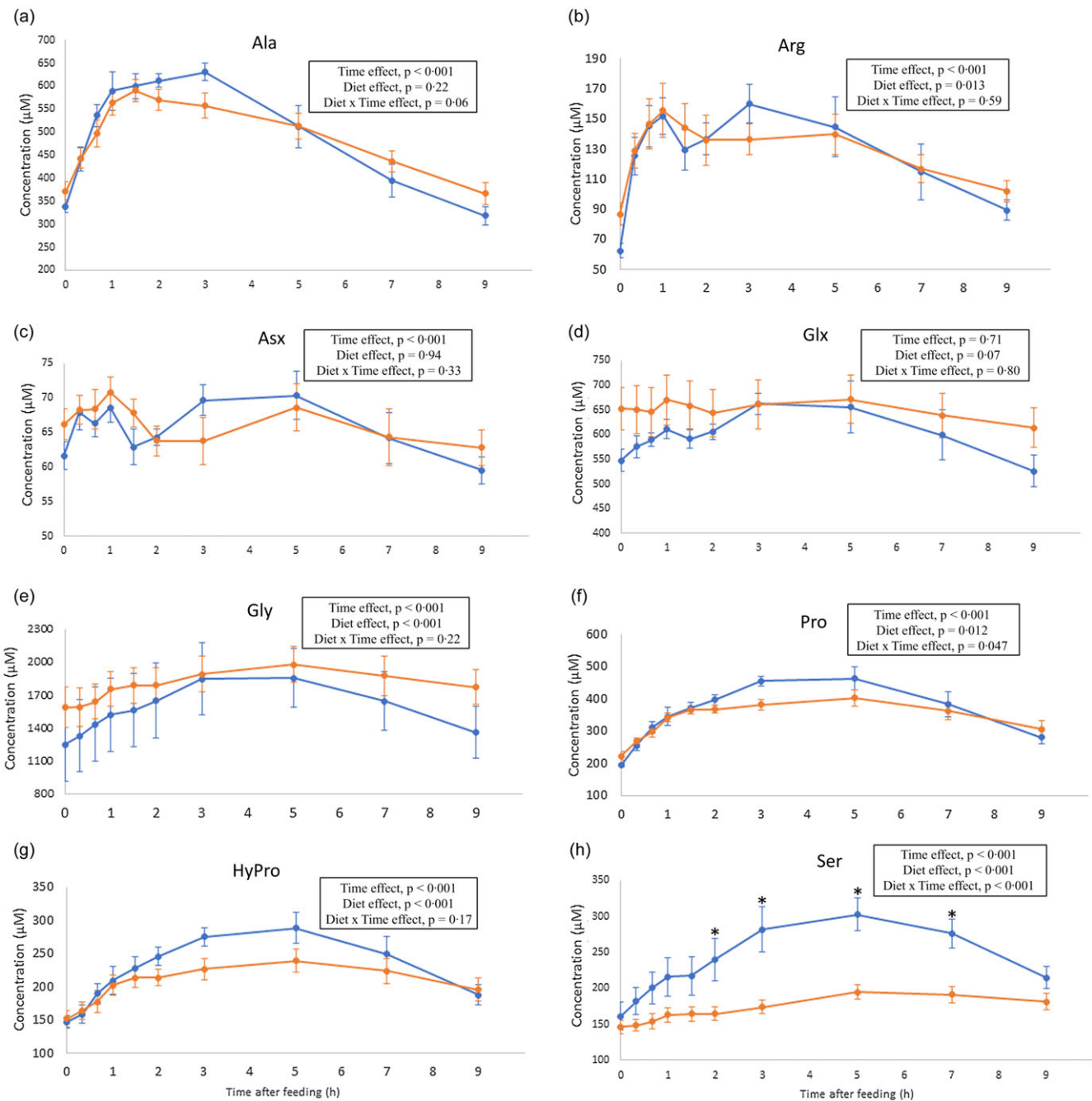


Fig. 3. Non-essential AA concentrations in plasma of pigs fed experimental diets containing water recovered proteins (WRP, blue, n 7) or greasy greaves recovered proteins (GGRP, orange, n 10) as the only dietary protein source. The data are presented as the mean \pm SEM. Non-essential AA are composed of Ala (a), Arg (b), Asx=Asn + Asp (c), Glx= Gln + Glu (d), Gly (e), Pro (f), HyPro (g) and Ser (h). The plasma AA concentrations were statistically analysed by a linear mixed model (lmerTest package) considering the factors 'diet', 'time' and their interaction, with pig as a random factor. When the interaction was statistically significant, a post hoc test was performed to establish the pairwise comparisons (Emmeans, Tuckey adjustment). A $P < 0.05$ was considered as significant and marked with an asterisk (curves).

short time^(13,38). Moreover, it has been demonstrated that heating collagen to 70 °C for 0.5 h induces conformational changes in proteins, modulating its affinity for pepsin and resulting in higher digestibility⁽⁴³⁾. Given the data available at the present time, it is therefore not possible to predict whether the TID of N and AA in

WRP and GGRP would be increased or decreased after cooking. As has been done for other food ingredients^(12,38), further work is needed to estimate the effects of processing on the nutritional quality of WRP and GGRP proteins and devise possible technological strategies to improve it.

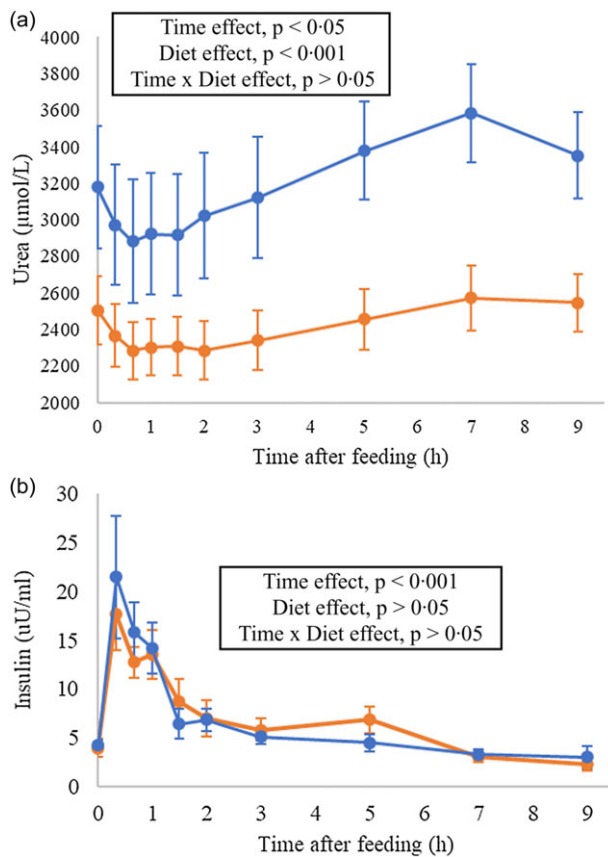


Fig. 4. Postprandial urea (a) and insulin (b) concentration in plasma of pigs fed experimental diets containing water recovered proteins (WRP, blue, n 7) or greasy greaves recovered proteins (GGRP, orange, n 10) as the only dietary protein source. The data are presented as the mean \pm SEM. The plasma concentrations were statistically analysed by a linear mixed model (*lmerTest* package) considering the factors 'diet', 'time' and their interaction, with pig as a random factor. A $P < 0.05$ was considered as significant.

The digestible indispensable amino acid score measurement classifies greasy greaves recovered protein as a good quality protein but solely for the adult population

The DIAAS of both WRP and GGRP ingredients was much below 75 for young children (undernourished or healthy), meaning that these protein sources are considered of low quality for these populations⁽⁷⁾. The low DIAAS values of WRP and GGRP result from a too low content of EAA, and especially of Trp that is the first-limiting AA, making both ingredients unbalanced proteins for young children. This results from the high collagen content, particularly for WRP, as collagen does not contain any Trp, leading even to a zero DIAAS value for beef collagen hydrolysate⁽³³⁾. The GGRP had a higher DIAAS (49% or 57% for undernourished or healthy children, respectively), consistently with an EAA composition closer to that of beef meat hydrolysate, for which a DIAAS of 63% has been reported⁽³³⁾. Combining GGRP with protein foods rich in Trp and His, that is, the only two EAA for which the DIAA reference ratio of GGRP is below 80% for healthy young children, would allow to obtain a protein mix of quality for this population.

For older children (> 3-years old), adolescents and adults, the DIAAS of WRP remains very low (10%), with Trp as the first-limiting EAA, unlike GGRP, whose DIAAS (74%) is close to the threshold of 75%, level at which a protein can be classified of good quality. This value indicates that the protein intake of GGRP needs to be 1.35 times higher than that of an 'ideal' protein (DIAAS 100%) to meet requirements. For this population, the first-limiting EAA is also Trp, but it can be underlined that for AAA, Thr and Val, the DIAA reference ratio values are above 100. These results are consistent with the DIAAS value calculated for a beef muscle hydrolysate, for which AA composition is close to GGRP, and for which a DIAAS of 81% has been previously reported for the same target population, with Trp as the first-limiting EAA⁽³³⁾. As a reminder, DIAAS values ranging from 80 to 99% have been determined for bovine meat depending on the cooking process⁽¹²⁾.

Overall, the present results indicate that GGRP is a good protein quality for older children, adolescents and adults, while this is not the case for WRP. Nonetheless, the WRP is remarkable for its content in His, 1.5 times higher than the recommendation for the older children, adolescents and adults. This co-product could therefore be used to supplement His-deficient plant proteins, such as black beans⁽⁶⁾.

The strategy of judiciously combining protein ingredients to compensate for the limitation or lack of certain EAA and reach DIAAS values >75%, as suggested by Bindari *et al.*⁽³³⁾, may be relevant for the GGRP ingredient, whose EAA composition is not so far from the nutritional requirements. Such a strategy has been successfully implemented in a previous study by combining of plant and animal proteins to achieve AA profiles adapted to the EAA needs of different target populations⁽⁴⁴⁾. It is therefore reasonable to assume that GGRP ingredient could contribute to the protein fortification of some plant foods that can be lacking of EAA such as Lys for cereals and sulphur amino acids for legumes⁽⁴⁵⁾.

It should be noted that some debate exists regarding the method of calculating the DIAAS, particularly regarding the method of calculating the AA profile (mg/g of protein), that is, either based on the CP content ($N \times 6.25$), as recommended by the FAO, or based on the total mass of AA residues⁽⁴⁶⁾. Using the latter methodology, the DIAAS values were somewhat higher, particularly for GGRP that achieves a DIAAS of 82% instead of 74% for the population of older children (> 3 years old), adolescents and adults (online Supplementary Table S2), in line with the N to collagen protein conversion factor of 5.55⁽⁴⁷⁾. However, the counterpart is that the protein concentration of the ingredient is assumed to be lower when considering a conversion factor of 5.55 instead of 6.25, meaning that a higher quantity of ingredient would be needed to meet the protein requirements.

Plasma amino acid concentrations were affected by the essential amino acid profile of the ingredients and by the microstructure of the diet

Plasma AA reached a maximum concentration at 3 h and 5 h after ingestion of the WRP and GGRP diets, respectively (Fig. 1), allowing to define both ingredients as slow protein sources

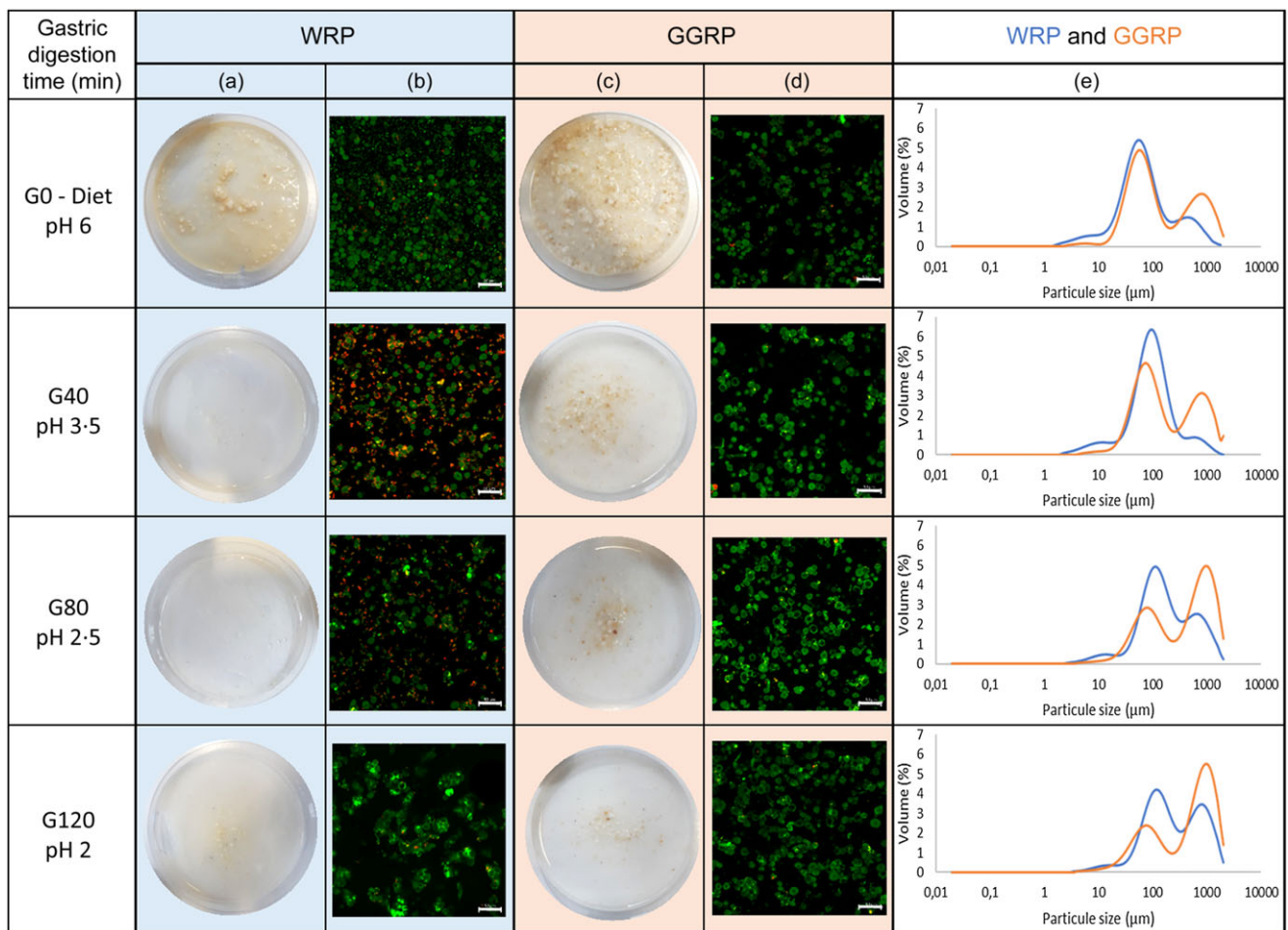


Fig. 5. Structural change of the experimental diets (WRP, blue; GGRP, orange) during *in vitro* semi-dynamic digestion at 0, 40, 80 and 120 min of digestion. Visual observations (a–c); confocal laser scanning microscopy images (b–d); particle size distribution (e). (b–d) Proteins are coloured in green and lipids in red; scale bar: 50 μm . WRP, water recovered proteins; GGRP, greasy greaves recovered proteins.

according to Boirie *et al.*⁽⁹⁾. This is consistent with a previous study in humans that reported a peak for plasma concentration of AA 3 h after eating bovine meat proteins⁽³⁸⁾. The slow AA release in the present study is likely due to the high collagen content of both GGRP and WRP ingredients, responsible for protein gelation leading to a high-diet viscosity, thus slowing down their gastric emptying rate and subsequently the rates of intestinal digestion and absorption⁽⁴⁸⁾. This was particularly true for GGRP diet, which had the highest viscosity throughout the *in vitro* digestion, in line with the slowest plasma AA appearance.

A higher level of plasma EAA concentration was observed along the entire postprandial period for GGRP diet compared with WRP diet (Fig. 1(c)), including at the preprandial time. This latter result suggests, during the adaptation period, a metabolic adaptation of the animals to the experimental diets, which contained a higher level of EAA in GGRP than in WRP. By the way, differences were similarly observed between the two diets at the preprandial time for Leu and Val (Fig. 2), for which the content was higher in GGRP than in WRP ($P < 0.001$). Such a fast metabolic adaptation was previously observed by Nørgaard *et al.*⁽⁸⁾ after 5 d adaptation.

In the present study and for most of the AA, the TID did not appear to be a determining factor for the plasma concentration of AA, as the TID did not differ between the diets ($P > 0.05$). Regarding Ala and Glx, whose concentration was similar in both diets, and despite a higher TID with the WRP diet than with the GGRP diet ($P = 0.013$ and 0.017 for Ala and Glx, respectively), no effect of diet on plasma concentration of these two AA was observed ($P > 0.05$; Fig. 3). This suggests that TID was not a determining factor in the plasma concentration of these two AA either. In contrast, for Arg, whose concentration was similar in both diets, an effect of the diet on plasma concentration was observed ($P = 0.013$). This could therefore reflect the effect of the TID of Arg, which was higher with the WRP diet than with the GGRP diet ($P = 0.009$), although the difference was small (+4%). Furthermore, it should be noted that the effect of diet on plasma Arg concentration was partially masked by the higher preprandial concentration of this AA in pigs fed GGRP (Fig. 3(b)). In addition, it should be borne in mind that plasma AA concentrations were determined in peripheral blood, that is, after the first extraction pass from the splanchnic area, which is an additional factor influencing plasma AA concentrations.

Table 5. Particle size and viscosity of the reconstituted WRP and GGRP diets and of the gastric digesta at 40, 80 and 120 min of digestion (G40, G80, G120)

	WRP						GGRP						P					
	Diet		G40		G80		G120		Diet		G40			G80		G120		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM		Mean	SEM	Mean	SEM	
$d_{0.5}$ (μm)	66.07	0.14	87.72	0.37	142.68	7.95	202.28	46.50	46.50	0.40	129.07	12.87	433.04	28.86	517.69	9.68	Diet, $P < 0.001$ Time, $P < 0.001$ Diet \times Time, $P < 0.001$	
$d_{4.3}$ (μm)	154.98	3.44	144.98	13.42	294.54	46.37	390.87	96.55	96.55	311.34	10.72	373.00	8.44	540.09	30.77	583.42	6.18	Diet, $P < 0.001$ Time, $P < 0.001$ Diet \times Time, $P > 0.05$
Viscosity (Pa.s at 10/s)	1.31	0.15	0.07	0.04	0.15	0.17	0.04	0.01	0.01	35.79	3.99	13.45	5.76	4.82	4.46	1.03	1.15	Diet, $P < 0.001$ Time, $P < 0.001$ Diet \times Time, $P < 0.001$

WRP, water recovered proteins; GGRP, greasy greaves recovered proteins. The data (mean \pm SEM) were statistically analysed by a linear mixed model (lmerTest package) considering the factors 'diet', 'time' and their interaction. A $P < 0.05$ is considered as significant.

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The plasma urea concentration was higher in WRP-fed pigs than in GGRP ones, even though the values remained in accordance with previous experiments conducted in growing pigs⁽²⁴⁾. The difference of plasma urea observed here, which is an indicator of the level of hepatic AA catabolism⁽⁴⁹⁾ and of EAA deficiencies^(50,51), was in line with the greatest imbalance of WRP in terms of EAA profile, compared with GGRP. Pigs fed WRP were thus in a more catabolic state than pigs fed GGRP, thus underlining that WRP cannot be solely fed to humans but needs to be completed with an adequate protein source.

Insulin, the main anabolic hormone stimulating postprandial AA utilisation for protein synthesis, notably in muscle^(52,53), was similar between diets, suggesting a similar anabolic stimulation for protein accretion following ingestion of both diets in pigs. The present plasma concentrations of insulin were in accordance with previous experimental data collected in growing pigs⁽²⁴⁾.

In conclusion, the present study has demonstrated that TID of N in WRP and GGRP was similar (84.2 ± 3.2 and 81.2 ± 2.3 %, respectively). The GGRP presents a nutritional protein quality adapted to child > 3-years old, adolescent and adult, unlike WRP. The minimum value that is recommended to make claims for protein quality of foods by FAO⁽⁷⁾ indicates that WRP protein concentrate needs to be supplemented with proteins with greater concentrations of EAA such as plant or animal proteins to fulfil the AA requirements of children, adolescents and adults.

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The authors declare none.

Supplementary material

For supplementary material/s referred to in this article, please visit <https://doi.org/10.1017/S0007114524001661>

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