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Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions

Effects of Different Protein Sources on Amino Acid Absorption and Plasma Appearance of Tryptophan, Large Neutral Amino Acids, and Tryptophan Metabolites in Pigs

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A B S T R A C T

Background: Absorption of tryptophan (TRP) across the gut epithelium is potentially modulated by competing large neutral amino acids (LNAAs), which could affect the appearance of TRP and its metabolites in the bloodstream.
Objectives: This study aimed to determine, in a growing pig model of an adult human, the absorption of TRP and other LNAAs from the gastrointestinal tract, and plasma appearance of TRP, LNAAs, and TRP metabolites, in response to dietary proteins varying in TRP content.
Methods: Pigs were adapted for 7 d to each of 4 diets that differed in their protein source and TRP content: *1*) alpha-lactalbumin (AL; 9.95 mg TRP/g diet DM), *2*) whey protein (6.59 mg TRP/g), *3*) casein (3.73 mg TRP/g), or *4*) zein (0.14 mg TRP/g). On day 8, pigs were euthanised after a 12-h fast (baseline), or 1, 2, 3, 4, or 6 h after they received a test meal consisting of 45 g protein, or a protein-free meal (n = 6 pigs at each time in each meal group). Tryptophan and LNAA absorption from the small intestine, and appearance of TRP, LNAAs, and TRP metabolites (melatonin, serotonin, kynurenine pathway metabolites), in the portal vein and systemic circulation, were determined.
Results: AL intake resulted in sustained elevated plasma TRP concentrations after an overnight fast. The amount of TRP absorbed was dose-dependently related to protein TRP content ($P = 0.028$), with fastest rates for pigs fed AL (371 mg/h). Portal and systemic plasma TRP, TRP/LNAA, and the TRP metabolites were highest ($P \leq 0.05$) after AL intake, and remained above baseline levels for ~4 h postprandially. Absorption rates of TRP correlated with postprandial plasma TRP and TRP metabolites ($P \leq 0.05$).
Conclusions: In adult humans, postprandial plasma TRP and TRP metabolite concentrations can likely be modulated by the TRP content of the meal.

Keywords: tryptophan, dietary protein, absorption, metabolites, pigs

Introduction

Tryptophan (TRP) is a dietary essential amino acid (AA). It is also a precursor for several biologically-active metabolites including serotonin and melatonin. TRP is metabolized through two major pathways, with the majority (~95%) through the hepatic kynurenine pathway where nicotinamide adenine dinucleotide is the major end-product, along with numerous other metabolites, some of which show biological activities [1]. Only ~5% of TRP is metabolized through the serotonin pathway in which serotonin and melatonin are produced. The majority (~90%) of serotonin production in the body occurs in the gastrointestinal tract (GIT). GIT-derived serotonin plays roles in GIT function, glucose homeostasis, lipid metabolism, and possibly also in metabolic disorders such as obesity and type 2 diabetes [2].

Abbreviations: 3HK, 3-hydroxykynurenine; AA, amino acid; AL, alpha-lactalbumin; BW, body weight; DM, dry matter; GIT, gastrointestinal tract; LNAA, large neutral amino acid; LOD, limit of detection; LOQ, limit of quantification; PF, protein free; TRP, tryptophan; WPI, whey protein isolate.

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Dietary protein is broken down into peptides and AAs in the stomach by gastric acid and pepsin, and by pancreatic and brush border proteases in the small intestine. Large neutral amino acids (LNAAs; leucine, isoleucine, phenylalanine, valine, tyrosine, threonine, methionine, and histidine) compete with TRP for absorption across the brush border of the small intestinal epithelium by the B^0 transporter [3]. On the basis of terminal ileal true digestibility values for different proteins, the majority of TRP appears to be absorbed, although the extent of absorption differs with the source of protein [4]. Competition between TRP and other LNAAs also influences the rate of absorption of these AAs [5,6]. TRP and other LNAAs also compete for uptake at other sites, such as the blood–brain barrier. The ratio of TRP concentrations compared with concentrations of remaining LNAAs (TRP/LNAA ratio) in the circulation is commonly used in human clinical trials as a measure of TRP availability to the brain [7].

Human clinical studies have reported an increase in postprandial plasma TRP and the TRP to LNAA ratio (TRP/LNAA) in response to high TRP intake, for example through administration of pure TRP [8–12], egg protein hydrolysate [13–15], or the high-TRP containing milk protein, alpha-lactalbumin (AL) [16–19]. No studies, however, have been performed to characterize the relationship between small-intestinal absorption kinetics and appearance of TRP and other LNAAs in blood in response to administration of proteins ranging in TRP content. Moreover, no studies have examined whether the ingestion of such proteins influences the flow of TRP through the serotonin pathway in the gut, terminating in the production of biologically-active molecules, such as melatonin.

Hence, this study was designed to determine in a pig model of the adult human, *1*) the absorption kinetics of TRP and LNAAs from the GIT; *2*) the appearance of TRP and LNAAs in the blood circulation, and TRP/LNAA ratios in the blood, and *3*) the appearance of bioactive TRP metabolites in the portal and systemic blood, most notably melatonin, in response to the ingestion of meals containing low (zein), medium (casein and whey) or high (AL) amounts of TRP. We hypothesize that the TRP content, relative to other LNAAs, of the meal will determine absorption, and plasma appearance, of TRP and LNAAs in a dose-dependent fashion, with the highest absorption and appearance of TRP, LNAAs, and melatonin, in response to the AL meal.

On the basis of previous studies suggesting that systemic TRP loading stimulates gut melatonin production/release in animals and humans [20,21], we further hypothesize that the appearance of melatonin in portal blood after meal ingestion would reflect the TRP absorption from the ingested proteins and post-meal melatonin synthesis in gut tissue. In addition, we also examined metabolites in the kynurenine pathway in blood, which derive principally from TRP metabolism in liver, as some appear to be active as signaling molecules at a variety of sites in the body [22]. The growing pig was used as an animal model for the adult human based on physiological similarities from mouth to the terminal ileum [23,24], and the pig being able to consume foods similar to that of humans.

Methods

Animals and housing

The study protocol has been published in detail [25]. Briefly, 180 nine-wk-old male pigs (PIC Camborough 46 x PICboar, mean ± SD body weight (BW): 22 ± 2 kg) were housed individually in metabolism crates in a room maintained at 22 ± 2°C with a 12 h:12 h light/dark (7.00 h:19.00 h) cycle for 8 d. There was no natural light inside the animal facilities. Light intensity from the artificial lightning in the facilities was measured at the front and middle of each cage (Lux & Foot Candle Light Meter, Digitech) at the height of the pig head, and values were averaged to determine the light intensity per cage. The mean light intensity was 155lux (range 23–256 lux) during the light cycle. Water was continuously available. The study was approved by the Animals Ethics Committee of Massey University (#17/05). The study was analyzed over in 6 periods with 30 pigs per period, in which each period contained 1 replicate of each diet by time combination.

Test meals

The meal fed to the animals on the test day consisted of 55.9 g of maltodextrin (Avondex 10, New Zealand Starch Limited) for the protein-free (PF) meal, or one of the following proteins (equivalent to 45 g crude protein): *1*) AL (Agropur Inc), *2*) whey protein isolate (WPI 8855, Fonterra Co-operative Group Limited), *3*) casein (Acid Casein 741, Fonterra Co-operative Group Limited), or *4*) zein (Zein W555025, Sigma-Aldrich New Zealand). The protein source or maltodextrin was mixed with 400 mL of tomato soup made from 20 g dehydrated tomato soup (Maggi Rich Tomato Soup Mix, Nestle New Zealand Limited). Protein content was tomato soup (~1 g), 150 g of PF cookies, and 1 g of the indigestible marker titanium oxide (TiO_2, Table 1). Diet and digesta TiO_2 contents were used to determine AA absorption [26]. Water (250 mL) was provided at 2 and 4 h postprandially. If consumption of the test meal was lower than 75 g dry matter (DM), data for that animal were removed from the analysis, because the amount of protein consumed was deemed to be negligible. The test meals given to the pigs did not contain the premix of vitamins and minerals used in the diets of the adaptation period to provide the same test meals given in a parallel but separate human study [27].

Experimental design

Upon arrival at the Animal Physiology Unit, Massey University, pigs (n = 180) were randomly allocated to the 5 dietary treatments. During an 8-day adaptation period, pigs were gradually adapted to the zein, casein, WPI, or AL test diets or a PF diet (n = 36 per diet group) [25]. These adaptation diets were identical to the test meals, except that they included a premix of vitamins and minerals not present in the test meals.

The pigs fed the zein diet were supplemented with 19 mg/kg DM TRP and 3 mg/kg DM lysine (based on TRP and lysine requirements for pigs 20–50 kg [28]) daily during experimental days 1–7, because these AAs are deficient in zein. The final zein test meal on day 8 was not supplemented. The pigs on the PF diet received the casein diet during experimental days 1–6 and received the PF diet during experimental days 7 and 8 (that is, the final 3 meals including the test meal). The PF diet was included in the study to determine the endogenous protein losses throughout the GIT lumen, to estimate the levels of endogenous AAs originating in the blood, and as a control condition to compare with the protein diets. All food refusals were recorded.

The daily ration was 90 g DM/kg of metabolic BW ($BW^{0.75}$) and was presented in 2 equal sized meals at 9.00 and 16.00 h. The last meal on day 7 was provided 12 h before receiving the

TABLE 1
Ingredient and nutrient composition of the test meal (g/kg dry matter, DM)[1]

Test meal	Casein	Alpha-lactalbumin	Whey protein isolate	Zein	Protein-free diet
Ingredients (g/kg DM)					
Casein[1]	247.9	—	—	—	—
Alpha-lactalbumin[2]	—	227.5	—	—	—
WPI[3]	—	—	228.6	—	—
Zein[4]	—	—	—	229.3	—
Maltodextrin[5]	—	—	—	—	229.0
Protein-free cookie	649.7	670.1	669	668.3	668.6
Tomato soup	93	93	93	93	93
Paracetamol[+]	4.7	4.7	4.7	4.7	4.7
Titanium dioxide	4.7	4.7	4.7	4.7	4.7
Determined nutrient content (g/kg DM)					
Starch	322	326	298	289	444
Total fat	111	112	116	111	104
Total dietary fiber	44	45	48	43	41
Ash	31	36	32	29	28
Crude protein	226	215	214	223	11
AA content of the test meal (g/kg diet)					
Tryptophan	3.73	9.95	6.59	0.14	0.17
Leucine	22.21	25.07	30.72	45.96	0.57
Isoleucine	12.04	13.30	12.78	9.79	0.21
Phenylalanine	12.61	10.07	8.60	16.44	0.43
Valine	14.00	8.98	10.61	8.23	0.33
Tyrosine	11.92	9.05	7.24	11.37	0.22
Threonine	9.61	11.89	10.71	6.71	0.18
Methionine	7.24	3.67	6.25	4.59	0.27
Histidine	5.53	6.13	3.73	2.59	0.08
Sum LNAAs excl. TRP	95.2	88.2	90.6	105.7	2.3
TRP/LNAA ratio	0.04	0.11	0.07	0.001	0.07

Abbreviations: AA, amino acid; DM, dry matter; LNAA, large neutral amino acid; TRP, tryptophan.
[6]The protein-free cookies were prepared by mixing maize starch (453 g/kg), sucrose (200 g/kg), margarine (290 g/kg), cellulose (50 g/kg), and baking powder (7 g/kg) before baking the dough at 190°C for 20 min.
[7]Added to allow calculation of stomach emptying rates in a separate but related study.
[1] Acid Casein 741, Fonterra Co-operative Group Limited, Palmerston North, New Zealand.
[2] Isolated α-lactalbumin, Saint-Hubert, Longueuil, Canada.
[3] WPI, whey protein isolate. WPI 8855, Fonterra Co-operative Group Limited, Palmerston North, New Zealand.
[4] Zein W555025, Sigma-Aldrich New Zealand, Auckland, New Zealand.
[5] Avondex 10, New Zealand Starch Limited, Auckland, New Zealand.

final morning meal on day 8 to keep the fasting times consistent between all pigs. On day 8, water was withdrawn 2 h before the final meal. All animals had ≥2 h of light before their final meal. The final meal was mostly consumed within 5 min of presentation or was removed after 15 min if the pig did not finish the meal.

On day 8, pigs were anesthetized 10 min before euthanasia at baseline ($T = 0$, before feeding), and 1, 2, 3, 4, and 6 h postprandially ($n = 6$ pigs per time point per meal). The samples collected at time 0 represented the AA and metabolite concentrations in the GIT and blood in a fasting state.

A veterinarian administered an anesthetic cocktail [0.12 mL/kg BW of Zoletil 100 (50 mg/mL), Ketamine (50 mg/mL) and Xylazine (50 mg/mL); Provet Pty Ltd] through an intramuscular injection. A second dose of the anesthetic cocktail (0.03 mL/kg BW) was administered intravenously into the lateral ear vein to ensure absence of pain response.

The abdominal cavity was opened, and blood samples were collected from the portal vein and left ventricle to determine differences in AAs and metabolites between blood that has not passed the liver (portal vein) and circulatory blood (left ventricle). Blood samples (10 mL) were collected in evacuated tube blood collection tubes containing EDTA (Becton Dickinson), and centrifuged at 2000 × g for 10 min at 4ºC, which likely resulted in platelet-poor plasma [29]. Blood plasma was aliquoted in cryotubes and stored at –80ºC until analysis. Then, the pigs were killed by an intra-cardial injection of sodium pentobarbitone (0.3 mL/kg BW of Pentobarb 300: Provet). The stomach (esophageal and pyloric sphincters), terminal ileum (last 30 cm of the small intestine), and the rectum were clamped before dissecting out the entire GIT. The terminal ileum was separated first, followed by the stomach and cecum. The remaining small intestine and colon were uncoiled. The chyme from the whole stomach and digesta from the small intestine were collected after flushing several times with a saline solution (0.9 g NaCl/L). Digesta from the cecum and colon were collected [26]. Feces excreted post-feeding were collected.

The samples were immediately frozen in dry ice, stored at –20°C, freeze dried, and ground in a coffee grinder before being manually sieved (1-mm screen opening size). A quarter of the freeze-dried stomach chyme and small intestinal digesta (without the terminal ileum) were pooled for AA analysis.

Chemical analysis

The four protein sources, maltodextrin, and the test meals were analyzed in duplicate for DM and crude protein (N × 6.25; Dumas method, using an elemental analyzer LECO) [30]. The meals, food refusals, and digesta (pooled terminal ileum, cecum, and colon) were analyzed for DM and TiO_2 [31]. Test meals, pooled digesta, and terminal ileal digesta were analyzed for standard AAs (using HCl hydrolysis, o-phthalaldehyde pre-column derivatization followed by reversed-phase HPLC), and for TRP (using alkali hydrolysis) [32].

Left ventricle and portal vein plasma free AAs (TRP, leucine, isoleucine, phenylalanine, valine, tyrosine, threonine, methionine, histidine) were analyzed by the AgResearch Analytical Laboratory, using the Pico-Tag method [33,34].

Left ventricle and portal vein plasma TRP metabolites [serotonin, melatonin, kynurenine, and 3-hydroxykynurenine (3HK)] were determined using LC-MS. To extract these metabolites, 200 μL plasma were thawed, and mixed with 5 ng of d5-TRP (Sigma-Aldrich) and 5 ng of d4-serotonin (Sigma-Aldrich) dissolved in a volume of 20 μL. D5-TRP was used as an internal standard for kynurenine and d4-serotonin was used as an internal standard for serotonin, 3HK, and melatonin. Two quality control plasma samples were spiked with 20 μL either "low" (L-trp: 100 μg/mL; serotonin: 500 ng/mL; melatonin: 1 ng/mL kynurenine: 50 μg/mL; 3HK: 1 μg/mL; d5-TRP: 250 ng/mL; d4-serotonin: 250 ng/mL) or "high" (L-trp: 500 μg/mL; serotonin: 2.500 μg/mL; melatonin: 5 ng/mL kynurenine: 250 μg/mL; 3HK: 5 μg/mL; d5-TRP: 250 ng/mL; d4-serotonin: 250 ng/mL) concentrations (relative to the standard curve) of each metabolite to calculate recovery rates. Recovery percentages were calculated by dividing the difference between the endogenous concentration of a metabolite in a non-spiked sample and the total concentration of a metabolite in the spiked samples (high or low), by the added concentration of a metabolite (Table 2). After adding the internal standards and/or spikes, samples were incubated at 4°C for 15 min, after which 800 μL of ice-cold chloroform/methanol (1:1 ratio) were added. Samples were then incubated at –20°C for 60 min, for protein and lipid precipitation. MilliQ water (400 μL) was added and samples were centrifuged at 12,800 × g for 15 min at 4°C (Eppendorf Centrifuge 5427 R). The organic layer (750 μL) was transferred into a glass vial, completely evaporated using a vacuum concentrator (Speed Vac Plus SC110A and Refrigerated Vapor Trap RVT400, Savant Instruments Inc.), and stored at –80°C until analysis. On the day of sample analyses, dried organic extracts were reconstituted in 100 μL of MilliQ water, and 2 μL were injected for analysis on a LC-MS/MS (Sciex). The samples were separated on a Hypersil GOLD 1.9 μm, 50 × 2.1 mm column (Thermo Fischer Scientific Inc.) at a temperature of 40°C, and a mobile phase flow rate of 700 μL/min. The mobile phases consisted of H_2O with 0.1% formic acid, and acetonitrile with 0.1% formic acid. External calibration was performed before and after every 25 samples and was performed by injection of a calibration mix of all metabolites in the range in which they were present in the sample. Quality control samples were also analyzed before and after every 25 samples. MultiQuant Software (Sciex, version 3.0.3) was used to integrate peak areas. Plasma melatonin, serotonin, kynurenine, and 3HK concentrations were calculated using metabolite to internal standard ratios. The limits of detection (LOD) were determined using the signal to noise ratio, and limit of quantification (LOQ) was defined as 3*LOD [35] (Table 2). Values below the LOD were set to 0. Although not all melatonin concentrations measured were above LOQ values, analyses including values in between the LOD and LOQ showed clear dose-dependent effects and as such were kept in the models. Values below LOD were set to zero, and values in between LOD and LOQ were included as their original values calculated using the melatonin to d4-serotonin (internal standard) ratio. As such, the melatonin results should be interpreted with some caution.

The recovery percentages of serotonin concentrations measured using LC-MS in this study were high (96%), and similar [36,37] or higher [38] than in other studies using LC-MS to measure serotonin. Melatonin recovery rates were lower than for serotonin (82%), and similar [39] or lower [40,41] than previously reported for plasma samples. Recovery rates of kynurenine (74%) were lower than for serotonin and melatonin but similar to

TABLE 2
Level of detection and quantification, recovery rates, median, and ranges of plasma serotonin, melatonin, kynurenine, and 3HK

	Level of detection	Level of quantification	Recovery rate		Average	Range
Serotonin (pmol/mL)	0.057	0.17	96 ± 4%	AL	113	3–2814
				Whey	69	4–494
				Casein	115	0–2894
				Zein	243	3–3109
				Protein-free	121	5–2230
Melatonin (pmol/mL)	0.043	0.13	82 ± 24%	AL	4.8	0–57.7
				Whey	0.57	0–4.64
				Casein	0.16	0–3.72
				Zein	0.13	0–1.53
				Protein-free	0.04	0–0.76
Kynurenine (nmol/mL)	0.0005	0.001	74 ± 5%	AL	16	1–67
				Whey	2.3	0.1–6.1
				Casein	0.8	0.4–1.8
				Zein	0.5	0.1–5.8
				Protein-free	0.48	0.2–0.8
3HK (pmol/mL)	0.45	1.34	63 ± 11%	AL	1446	127–3349
				Whey	154	7–340
				Casein	28	1–57
				Zein	27	8–384
				Protein-free	20	6–43

Abbreviation: 3HK, 3-hydroxykynurenine.

previously recorded recovery rates for kynurenine [42], although some other studies have found recovery rates close to 100% [38,43]. In terms of 3HK, recovery rates were relatively low (63%), and lower than previously reported [42,43].

Calculations and statistical analysis

LNAA concentrations were calculated by summing the blood plasma AA concentrations or the absorption (disappearance from small intestine) values for leucine, isoleucine, phenylalanine, valine, tyrosine, threonine, methionine, and histidine. Statistical analyses were conducted using SAS (version 9.4; SAS Institute Inc.).

The apparent and true absorption of TRP and LNAAs in the lumen were calculated as follows:

$$\text{Apparent AA}_i\text{ disappeared (mg)} = \text{AA}_i\text{ content}_{\text{Meal}} - \left(\text{Apparent AA}_i\text{ unabsorbed} + \text{AA}_i\text{ content}_{\text{Refusal}}\right)$$

$$\text{True AA}_i\text{ disappeared (mg)} = \text{Apparent AA}_i\text{ disappeared} - \text{AA}_i\text{ endogenous}$$

$$\text{Apparent AA}_i\text{ unabsorbed (mg)} = \text{AA}_{i\text{ pooled digesta}} + \text{AA}_{i\text{ terminal ileal digesta}} + \text{AA}_{i\text{ released into the large intestine}}$$

$$\text{AA}_i\text{ released into the large intestine (mg)} = \left(\text{TiO}_2\text{ content}_{\text{Cecal digesta}} + \text{TiO}_2\text{ content}_{\text{Colonic digesta}}\right) \times \text{AA}_i\text{ content}_{\text{Terminal ileal digesta}} \,/\, \text{TiO}_2\text{ content}_{\text{Terminal ileal digesta}}$$

$$\text{AA}_i\text{ endogenous (mg)} = \text{AA}_{i\text{ pooled digesta of pigs fed the protein-free diet}} + \text{AA}_{i\text{ terminal ileal digesta of pigs fed the protein-free diet}} + \text{AA}_{i\text{ released into the large intestine of pigs fed the protein-free diet}}$$

$$\text{Apparent AA}_i\text{ absorption (\%)} = \left(\text{AA}_i\text{content}_{\text{Diet}} - \left(\text{Apparent AA}_i\text{ unabsorbed} + \text{AA content}_{\text{refusal}}\right)\right) / \left(\text{AA}_i\text{content}_{\text{Diet}} - \text{AA content}_{\text{refusal}}\right) \times 100$$

An analysis of covariance model was used to determine the effects of dietary treatment, postprandial time (up to quintic order) and the interaction between dietary treatment with time on TRP and LNAAs absorption from the gut lumen, and the appearance of plasma TRP, LNAAs, TRP metabolites (melatonin, serotonin, kynurenine, and 3HK). The selected polynomial model for each response variable was chosen after comparing higher order models with reduced order models (that is, removing predictors that did not affect the response variable) using the log-likelihood ratio test. The light intensity differed across individual cages. Thus, light intensity was included in each response variable as a covariate and remained in the final model when it was statistically significant. The model diagnostics of each fitted model were tested after combining the PROC UNIVARIATE and the ODS GRAPHICS procedures of SAS. When required, a transformation (natural log, square root) was conducted to achieve normal distribution and/or homogeneity of variance. Transformed values from the model were transformed back to calculate magnitudes of increase from baseline concentrations. Baseline values were transformed to achieve homogeneity of variance, and analyzed using a 1-way analysis of variance model, with dietary treatment as the fixed factor. If one of the response variables was significant, post hoc testing was conducted with Tukey-adjustments for multiple comparisons. Probability values of $P \leq 0.05$ were considered of statistical significance.

Spearman's correlation analysis was conducted between different variables using the PROC CORR of SAS statement. To correct postprandial values for potential confounding effects of baseline values in the correlation analyses, absorption values and plasma concentrations were corrected for mean baseline values of the corresponding diet. Correlations were considered relevant if $r \geq 0.5$ and $P \leq 0.05$.

Results

One of the pigs on the PF meal was removed from the study because of illness unrelated to the treatments. Ten pigs were excluded from analyses because of low intake of the test meal [AL/1 h; AL/4 h; casein/2 h; PF/3 h; PF/6 h; zein/ 1 h; zein/2 h; zein/3 h (2 pigs); zein/4 h]. Mean intake (± SD) of the test meals on the final day was 190 ± 55.4 g DM, ranging between 162.7 ± 73.0 g for the zein meal and 202.9 ± 32.7 g for WPI (Supplemental Table 1). Average BW at the end of the study was 26 ± 2.5 kg and was lower for the pigs on the zein diet compared with the remaining pigs because of a lower daily intake (Supplemental Table 1). AA intake varied between meals for TRP [range: 26 mg (zein)–2321 mg (AL)] and TRP/LNAA ratio [range: 0.001 (zein)–0.112 (AL); Table 3], whereas the intake of LNAAs (sum) was similar across all meals [range: 19,360 (zein)–21,083 (casein) mg].

Digestibility of TRP and LNAAs from the small intestinal lumen

There were significant interaction effects of meal type by time for the absolute true digestibility of TRP ($P = 0.028$; Figure 1) and LNAAs ($P = 0.009$), and the TRP/LNAA ratio ($P < 0.001$), and linear patterns of absorption were observed across the 6 h postprandial period for TRP and the LNAAs. TRP absorption was dose-dependent on the TRP content in the protein: true TRP absorption rate was highest for the pigs fed the AL meal (371 mg/h), followed by WPI (278 mg/h) and casein (95 mg/h). LNAA absorption rates were higher for the pigs fed WPI (3815 mg/h), compared with pigs fed with zein (2676 mg/h), casein (1936 mg/h), and AL (2594 mg/h). TRP/LNAA absorption ratios increased most after AL and remained stable over the 6-h postprandial period for AL and casein but decreased for WPI. The true ileal digestibility of TRP and LNAAs for each protein source over time is shown in Table 4.

Portal vein and left ventricle plasma TRP and LNAA concentrations, and TRP to LNAA ratio

Portal and left ventricle baseline plasma TRP concentrations ($t = 0$ h) were higher in the pigs fed AL compared with pigs fed with the other meals, and TRP/LNAA ratio baseline concentrations were higher in AL, WPI, and PF compared with the casein and zein-fed pigs ($P \leq 0.05$; Table 5).

There were significant ($P \leq 0.05$) interaction effects of meal type and time for portal vein and left ventricle plasma TRP and LNAA concentrations, and the plasma TRP/LNAA ratios (Figure 2). Increases from baseline in portal vein and left ventricle plasma TRP concentrations occurred mainly during the first hour after consumption of the AL (portal: +4.5-fold; left ventricle: +4.1-fold), whey (portal: +4.4-fold; left ventricle: +2.8-fold), and casein (portal: +2.3-fold; left ventricle: +1.9-

TABLE 3
Determined AA intake (mg) at the test meal

	Alpha-lactalbumin	Whey protein isolate	Test meal		
			Casein	Zein	Protein-free diet
Tryptophan	2321 ± 464	1459 ± 343	826 ± 213	26 ± 6	38 ± 8
Leucine	5847 ± 1169	6806 ± 1600	4920 ± 1269	8419 ± 1873	124 ± 26
Isoleucine	3101 ± 620	2831 ± 666	2668 ± 688	1793 ± 399	46 ± 10
Phenylalanine	2347 ± 469	1904 ± 448	2794 ± 721	3011 ± 670	95 ± 20
Valine	2093 ± 419	2351 ± 2351	3102 ± 800	1508 ± 1509	49 ± 10
Tyrosine	2110 ± 422	1604 ± 377	2640 ± 681	2082 ± 464	47 ± 10
Threonine	2773 ± 555	2372 ± 558	2130 ± 550	1228 ± 273	38 ± 8
Methionine	856 ± 171	1385 ± 326	1604 ± 414	841 ± 187	59 ± 12
Histidine	1430 ± 286	826 ± 194	1225 ± 316	475 ± 106	17 ± 4
Sum LNAAs	20,560 ± 4112	20,083 ± 4722	21,083 ± 5439	19,360 ± 4309	475 ± 100
TRP/LNAA ratio	0.112	0.073	0.039	0.001	0.080

Abbreviations: AA, amino acid; LNAA, large neutral amino acid; TRP, tryptophan.
Values are mean ± SD.

fold) meals, after which concentrations stabilized for ~3–4 h, then declined toward baseline values at 6 h (Figure 2; Supplemental Table 2). Portal vein and left ventricle plasma LNAA concentrations increased 2–3-fold in the first hour after consumption of all protein meals, but decreased (~–12%) after the PF meal.

As a result of the meal-related change in plasma TRP but only minor meal effects on LNAA concentrations, the plasma TRP/LNAA ratio followed a trend similar to plasma TRP concentrations. As such, there was an increase in ratios 1 h post-feeding followed by a 3- to 4-h plateau, which was most prominent after feeding AL [portal vein: peak of 0.054 ± 0.003 (mean ± SE) at 3 h; left ventricle: peak of 0.047 ± 0.002 at 3 h].

Portal vein and left ventricle plasma melatonin, serotonin, kynurenine, and 3HK concentrations

Baseline concentrations ($t = 0$ h) of portal and left ventricle plasma kynurenine and 3HK were higher in the AL pigs compared with pigs consuming the other meals ($P \leq 0.05$; Table 5).

There were significant ($P \leq 0.05$) interaction effects of meal with time for left ventricle and portal vein plasma melatonin, kynurenine, and 3HK, and portal vein serotonin concentrations (Figure 3). Plasma melatonin concentration increased 6.7-fold (portal vein) and 13-fold (left ventricle) from baseline after AL consumption during the first 4 h post-feeding and increased 2.2-fold (portal vein) and 3.6-fold (left ventricle) in the first 4 h after administration of WPI, but remained unchanged after the casein, zein, and PF meals (Supplemental Table 2).

Left ventricle and portal vein plasma kynurenine concentration increased ($P \leq 0.05$) steadily for 3–4 h postprandially after feeding AL or WPI, with 3.3-fold (portal vein; 4 h postprandially) and 3.6-fold (left ventricle; 3 h) increases from baseline after feeding AL, and 2.6-fold (portal vein; 4 h) and 3.1-fold (left ventricle; 3 h) increases from baseline after feeding WPI. Plasma concentrations of 3HK increased for 6 h postprandially, with 5.3-fold (portal vein; 6 h postprandially) and 2.8-fold (left ventricle; 6 h) increases from baseline after WPI; and 2.2-fold (portal vein; 6 h) and 2.1-fold (left ventricle; 6 h) increases from baseline after AL. No effects of time occurred for left ventricle or portal vein plasma serotonin concentration in response to any of the test meals.

Correlations between intake, absorption, and appearance in blood circulation of TRP, LNAAs, and TRP metabolites

Baseline concentrations were subtracted from all postprandial blood plasma results to overcome confounding effects. The amounts of TRP ingested and the ratio of TRP/LNAA ingested were positively correlated with the amount of TRP absorbed at 1, 2, 3, 4, and 6 h ($n = 36$ pigs per time point) postprandially ($r \geq 0.90$, $P < 0.001$), but the intake of LNAAs was not significantly correlated with the amount of LNAAs absorbed (Table 6). Although both TRP intake and TRP/LNAA ratio of the meal were positively associated with plasma TRP (TRP intake: $r \geq 0.78$; $P < 0.001$; TRP/LNAA ratio intake: $r \geq 0.61$, $P < 0.001$) concentrations and TRP/LNAA ratio (TRP intake: $r \geq 0.46$, $P < 0.001$; TRP/LNAA ratio intake: $r \geq 0.52$, $P < 0.001$), only TRP intake was consistently positively correlated with plasma concentrations of some of the metabolites ($r \geq 0.49$, $P < 0.001$). Correlations between TRP intakes and plasma metabolite concentrations were generally strongest 2–3 h after meal ingestion.

Absorption of TRP was positively correlated with plasma TRP concentrations in the portal vein ($r = 0.47$) and left ventricle ($r = 0.67$), and plasma melatonin concentrations ($r = 0.46$) and kynurenine ($r = 0.49$) in the left ventricle (all $P < 0.05$; Table 7). TRP absorption was not correlated with plasma TRP/LNAA ratios or plasma TRP metabolite plasma concentrations other than left ventricle plasma melatonin and kynurenine concentrations.

Discussion

To our knowledge, this is the first study that has determined the rate of absorption of TRP and the remaining LNAAs, and the TRP/LNAA absorption ratio from the small intestine in response to protein meals with a wide range of TRP content and TRP/LNAA ratio. Furthermore, total digesta collection over time provides unique insights into both the absolute absorption of AAs, as well as rates of AA uptake over time.

This study establishes that the small intestinal absorption of TRP, postprandial plasma TRP concentrations, TRP/LNAA ratios, and TRP metabolite (though not serotonin) concentrations in general increase dose-dependently with the TRP content of the meal. Consequently, ingestion of the protein meal with the highest TRP content, AL, resulted in the highest postprandial

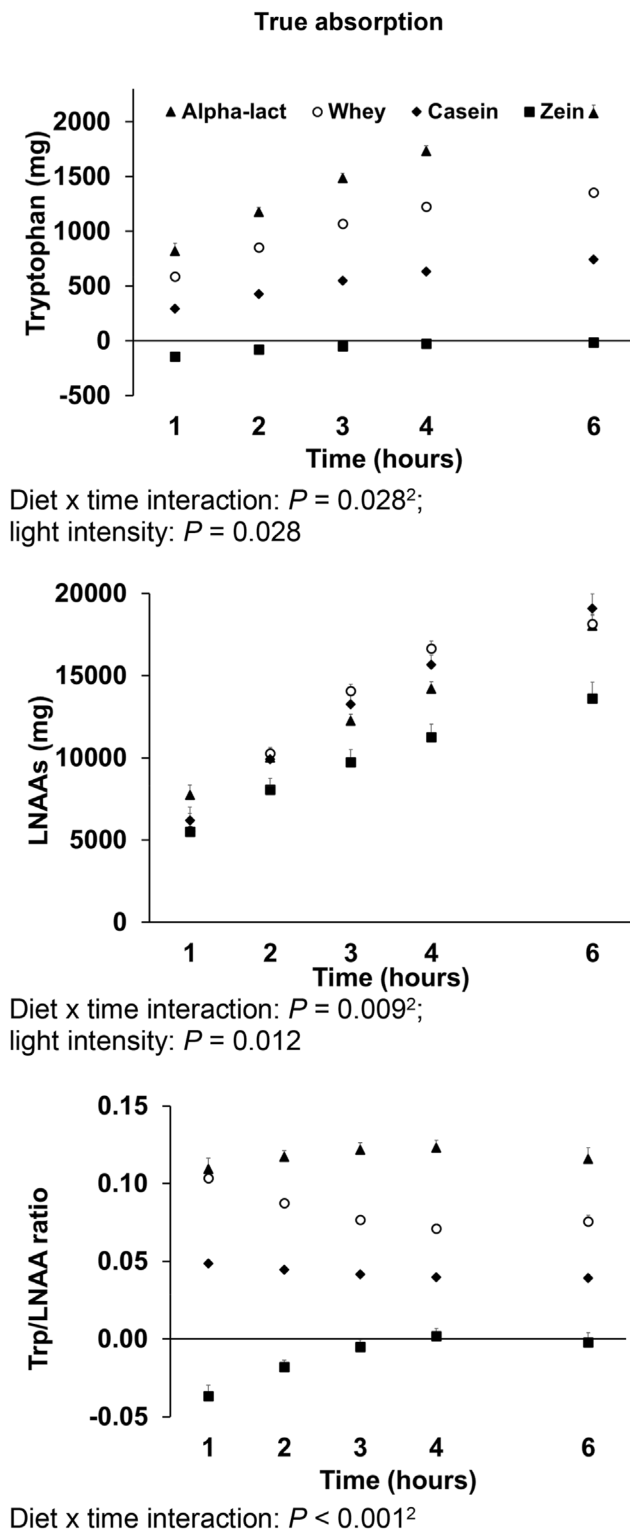

FIGURE 1. Cumulative true small intestinal absorption of tryptophan (TRP), large neutral amino acids (LNAAs), and the TRP to LNAA absorption ratio of growing pigs fed meals containing zein, casein, whey, and alpha-lactalbumin protein over 6 h post-feeding. Values are means ± SEM. [2]Quadratic effects for the time factor. Light intensity was used as a covariate in the model and reported when statistically significant.

plasma TRP/LNAA ratio and some TRP metabolite concentrations (Figure 3). Additionally, plasma TRP and TRP metabolite concentrations (except serotonin) remained elevated after a 12-h overnight fasting period (Figures 2 and 3) after the AL meal. Whereas absorption of TRP from the small intestine increased linearly over time, plasma TRP (although not consistently so) and TRP metabolite concentrations increased mostly in the first 1–3 h before returning to baseline values by 6 h, resulting in only weak to moderate positive correlations between these outcomes. These findings support our hypothesis that the appearance of TRP, LNAAs, and TRP metabolites over time in the blood circulation, in response to different proteins with different AA compositions, is influenced by the amount of TRP and LNAAs absorbed from the small intestine.

Small intestinal absorption of TRP and LNAAs

This study established that the absolute true absorption of TRP and other LNAAs increased in a generally linear fashion during the 6-h postprandial period (Figure 1). However, the amounts of TRP absorbed varied among the meals, with highest amount of TRP absorbed in the AL-fed pigs, and lowest absorption in the zein-fed pigs. The TRP content of the meal thus was reflected in how much TRP was absorbed from the small intestine and how much TRP appeared in the circulation—supported by strong correlations between TRP intake and the amount of TRP absorbed. Although less pronounced, a similar pattern was observed for the remaining LNAAs—with lowest amounts of LNAA absorption in the zein-fed pigs.

Although true amount of LNAA absorption differed between AL, whey, and casein-based meals, true rates of TRP absorption were more divergent between these meals, resulting in diet-related differences in the ratio of TRP/LNAA absorption (Figure 1). Like TRP absorption, TRP/LNAA absorption rates were highest after the AL meal. Interestingly, postprandial TRP/LNAA absorption showed a decreasing trend after WPI, likely the result of its relatively high content of LNAAs relative to TRP, and perhaps, increased competition for TRP to be transported across the epithelial membrane of the small intestine, as all LNAAs use the B^0 transporter [3,44]. Indeed, we found positive correlations between the TRP/LNAA ratio of the meal intake—which can be interpreted as a measure for TRP competition with other LNAAs for absorption from the small intestine—and TRP true absorption from the GIT.

Of note, physiochemical differences of the proteins in the small intestinal lumen could have further affected absorption. For example, dietary caseins have a relatively slow transit of AAs to the upper intestinal lumen [45], because of clot formation in the stomach [46], whereas whey proteins remain in a dissolved state and move more quickly through the stomach, resulting in a more rapid delivery of AAs to the bloodstream [47].

Appearance of TRP and LNAAs in the portal and left ventricle blood plasma

Most studies in humans [16,18,48] and pigs [49,50] have reported plasma TRP/LNAA ratios at a single postprandial time point. Postprandial plasma effects of a standardized amount of protein at multiple timepoints, after a strict overnight fasting period, have not been measured in previous studies. Using a standardized meal protocol in the current study allowed measurements of baseline TRP and LNAA plasma concentrations after a 12-h overnight fast. It is remarkable that the fasting plasma concentration of TRP was higher in the AL-fed pigs compared with the pigs from the other diet groups. The meal effects on baseline TRP concentrations indicate that the high small intestinal absorption of TRP, through

TABLE 4
True digestibility values (%) of TRP and remaining LNAAs in the test meals at each postprandial time point

Time (h)	Alpha-lactalbumin		Whey protein isolate		Casein		Zein	
	TRP (%)	LNAAs (%)	TRP (%)	LNAAs (%)	TRP (%)	LNAAs (%)	TRP (%)	LNAAs (%)
1	39.7 ± 5.8	43.0 ± 6.2	50.6 ± 16.6	36.6 ± 22.3	38.1 ± 8.2	31.0 ± 7.1	0	23.5 ± 8.0
2	61.0 ± 21.8	52.9 ± 9.4	58.1 ± 5.3	48.2 ± 8.6	68.5 ± 26.8	60.5 ± 16.9	0	47.7 ± 18.3
3	68.3 ± 11.4	68.3 ± 8.3	76.7 ± 7.1	74.6 ± 7.7	69.0 ± 6.7	64.7 ± 8.5	0	46.1 ± 7.3
4	86.5 ± 10.1	77.0 ± 10.4	87.4 ± 7.2	84.1 ± 10.8	80.4 ± 8.7	77.8 ± 10.3	0	51.8 ± 16.7
6	96.9 ± 2.1	95.5 ± 3.5	95.9 ± 3.6	93.3 ± 7.1	93.3 ± 6.7	92.5 ± 5.6	0	75.9 ± 12.4

Abbreviations: AA, amino acid; LNAA, large neutral amino acid; TRP, tryptophan.
Values are mean ± SD. Values are calculated as a percentage of the AA intake (minus refusals).

TABLE 5
Baseline plasma concentrations (transformed values) of tryptophan (TRP), large neutral amino acids (LNAAs), TRP/LNAA ratio, and TRP metabolites in the portal vein and left ventricle

	Alpha-lactalbumin	Whey protein	Casein	Zein	Protein-free diet
Portal vein					
Log tryptophan (nmol/mL)[1]	3.64 ± 0.92[a]	2.69 ± 0.20[b]	2.51 ± 0.27[b]	2.33 ± 0.24[b]	2.34 ± 0.25[b]
Log LNAAs (nmol/mL)[1]	7.55 ± 0.23[a]	6.79 ± 0.16[b,c]	7.16 ± 0.11[c]	7.06 ± 0.15[c]	6.49 ± 0.16[b]
TRP/LNAA ratio[1]	0.013 ± 0.001[a]	0.017 ± 0.002[b]	0.008 ± 0.001[c]	0.008 ± 0.002[c]	0.015 ± 0.002[a,b]
Log serotonin (pmol/mL)	3.54 ± 0.97	2.74 ± 0.93	3.76 ± 2.23	2.78 ± 0.68	2.96 ± 0.68
Log melatonin (pmol/mL)	−0.31 ± 0.53	−2.17 ± 1.40	−1.12 ± 1.91	−1.87 ± 1.68	−2.76 ± 1.38
Log kynurenine (nmol/mL)[1]	1.70 ± 0.83[a]	−0.08 ± 1.21[b]	−0.61 ± 0.18[b]	−0.59 ± 0.34[b]	−0.82 ± 0.35[b]
Log 3HK (pmol/mL)[1]	6.59 ± 1.00[a]	3.88 ± 1.21[b]	2.56 ± 1.42[b]	3.25 ± 0.21[b]	2.72 ± 0.48[b]
Left ventricle					
Log tryptophan (nmol/mL)[1]	2.98 ± 0.21[a]	2.59 ± 0.11[b]	2.28 ± 0.21[b,c]	2.13 ± 0.29[c]	2.23 ± 0.25[c]
Log LNAAs (nmol/mL)[1]	7.33 ± 0.14[a]	6.67 ± 0.09[b]	7.09 ± 0.07[c]	7.02 ± 0.08[c]	6.48 ± 0.11[d]
TRP/LNAA ratio[1]	0.025 ± 0.02[a]	0.017 ± 0.001[a]	0.01 ± 0.002[a]	0.009 ± 0.003[a]	0.016 ± 0.002[a]
Log serotonin (pmol/mL)	3.39 ± 1.92	4.33 ± 1.48	3.86 ± 1.92	4.17 ± 1.01	3.83 ± 1.26
Square root melatonin (pmol/mL)	0.34 ± 0.48	0.09 ± 0.1	0.04 ± 0.08	0.004 ± 0.004	0.10 ± 0.23
Log kynurenine (nmol/mL)[1]	1.66 ± 0.95[a]	0.24 ± 0.39[b]	−0.67 ± 0.23[b,c]	−0.67 ± 0.37[b,c]	−0.79 ± 0.41[c]
Log 3HK (pmol/mL)[1]	6.73 ± 1.05[a]	4.54 ± 0.78[b]	3.30 ± 0.37[c]	3.25 ± 0.30[c]	3.0 ± 0.38[c]

Abbreviation: 3HK, 3-hydroxykynurenine.
Baseline concentrations of plasma TRP, LNAAs, TRP/LNAAs, and TRP metabolites. Logarithmic (portal melatonin, serotonin, kynurenine, and 3HK) and square root (left ventricle melatonin) transformations were used to fulfill model assumptions. Results were analyzed using a one-way analysis of variance, with diet as the fixed factor. Post hoc testing was conducted with Tukey-adjustments for multiple comparisons.
[a,b,c,d] A different letter indicates a statistical difference between baseline values within a row. Values are mean ± SD.
[1] Main effect of diet was $P < 0.05$.

administration of protein high in TRP content, increases plasma TRP concentrations not only postprandially, but also at a more sustained level. Although a meal-effect remained for postprandial plasma concentrations of TRP and ratios of TRP/LNAA, it should be noted that the increased baseline TRP concentration contributed to the high postprandial concentrations of TRP and TRP/LNAA concentrations.

High fasting TRP concentrations in the AL pigs may indicate that, although excess AAs are thought to be either directly oxidized or converted to glucose and triglycerides, an abundance of TRP is not entirely cleared from the blood stream within 12 h of administration. Our fasting values cannot be compared with other pig studies modifying TRP intake because these usually allow ad libitum feed intake [49–54]. Only 2 human clinical studies using prolonged TRP supplementation (instead of an acute study protocol) were found, and these did not detect an effect of TRP supplementation on fasting plasma TRP concentrations [8,15]. These studies used lower amounts of TRP (≤1.8 g/d), suggesting that relatively large amounts of TRP administration are required to modify fasting plasma TRP concentrations.

One human clinical trial [19] reported dose-dependent increases of TRP concentrations and TRP/LNAA ratios in peripheral plasma postprandially over time for 6 h after administration of 40 g AL, wheat gluten, zein, and cornstarch. Similar results were found here: the ingestion of the meal containing AL produced a substantial rise in portal plasma TRP concentrations between 1 and 4 h postprandially. Post-meal increases in plasma TRP were also large after ingestion of the WPI meal, but peak values were much smaller than those attained after AL ingestion. Casein ingestion elicited a small rise in plasma TRP, whereas the PF and zein-containing meals produced no increases in plasma TRP. The relative increases in plasma TRP after the ingestion of AL, WPI, and casein reflect the relative proportion of TRP present in each protein source [55].

Furthermore, the largest increase in postprandial TRP plasma concentrations was observed in the first 60 min, whereas plasma TRP/LNAA ratios continued to increase for ~3 h postprandially. The results of the current study showed that the linear increase of true TRP and LNAA absorption over the 6 h postprandial was not reflected in the appearance pattern of TRP, LNAAs, and TRP/

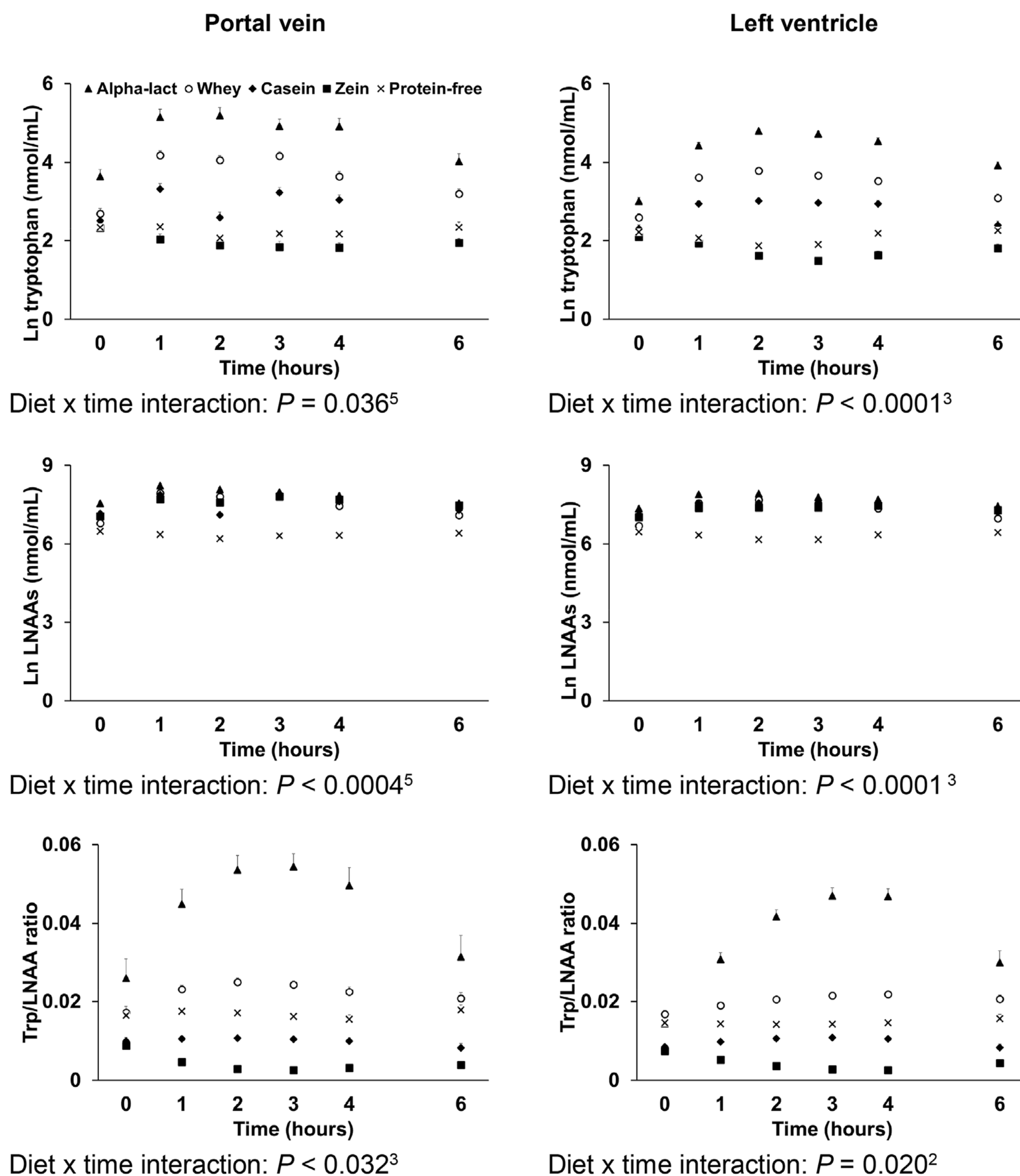

FIGURE 2. Tryptophan (TRP), large neutral amino acid (LNAA) concentrations, and the TRP to LNAA ratio in portal vein and left ventricle blood plasma of growing pigs fed meals containing zein, casein, whey, and alpha-lactalbumin protein or a protein-free diet. Logarithmic transformations were used to fulfill model assumptions for tryptophan and LNAA concentrations. Values are means ± SEM. Chosen models included [2]quadratic, [3]cubic, or [5]quintic effects for the time factor.

LNAAs in the plasma, which mostly showed large increases in the first two postprandial hours after which concentrations returned toward baseline concentrations. Therefore, correlations between absorption of TRP and appearance of TRP, TRP/LNAA, and TRP metabolites in the plasma, corrected for baseline values, were mostly statistically nonsignificant.

Appearance of TRP metabolites in the portal and left ventricle blood plasma

At baseline (0 h), and throughout the subsequent 6-h period, kynurenine and 3HK concentrations were highest in animals ingesting AL, intermediate in animals ingesting WPI, and lowest in animals ingesting casein, zein, and the PF meals. These differences paralleled the differences in TRP content of the meals, and TRP absorption. Kynurenine metabolites are generated in the liver, with their production varying directly with plasma TRP concentrations [22]. Although we did not measure additional kynurenine pathway metabolites, the results indicate that it would be useful to do so in a future study. For example, kynurenic acid and quinolinic acid are derived from kynurenine and are active at receptors for the AA neurotransmitter glutamate in brain [22]. Pharmacological increases in blood TRP and kynurenine concentrations are known to elevate kynurenic and quinolinic acids levels in brain [56,57]. Such might also be the case when proteins are ingested that produce significant increases in plasma TRP and kynurenine concentrations. If so, then dietary variations in TRP supply to the blood, and ultimately the brain, might potentially affect the synaptic activity of neurotransmitters glutamate as well serotonin.

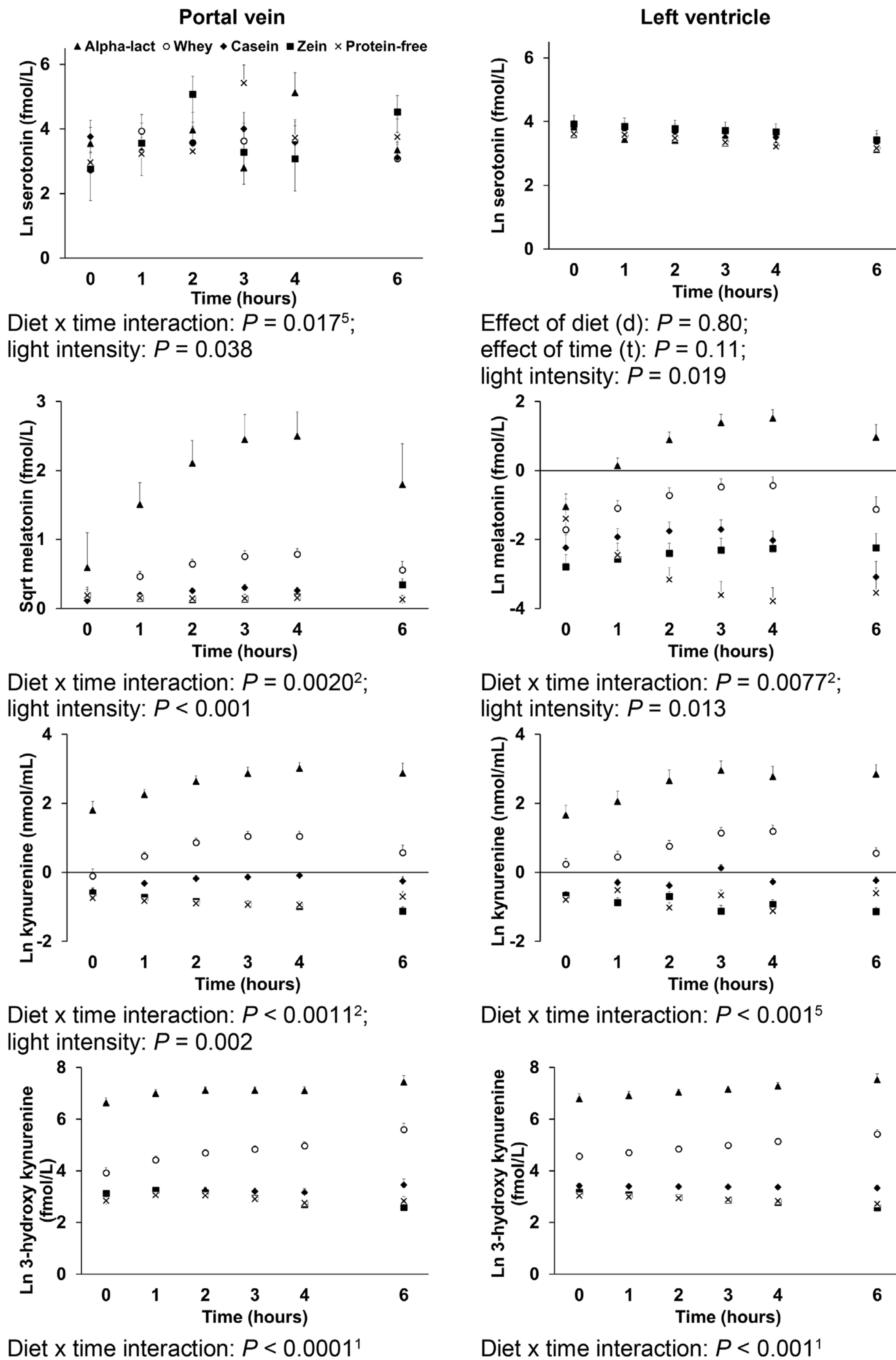

FIGURE 3. Melatonin, serotonin, kynurenine, and 3HK concentrations in portal vein and left ventricle blood plasma of growing pigs fed meals containing zein, casein, whey, and alpha-lactalbumin protein or a protein-free meal. Logarithmic (serotonin, left ventricle melatonin, kynurenine, and 3HK) and square root (portal melatonin) transformations were used to fulfill model assumptions. Values are means ± SEM. Chosen models included [1]linear, [2]quadratic, or [5]quintic effects for the time factor. Light intensity was used as a covariate in the model and reported when statistically significant. 3HK, 3–hydroxykynurenine.

TABLE 6

Correlations (*r* values) between ingested and absorbed tryptophan (TRP), large neutral amino acids (LNAAs), and TRP/LNAA ratios, and plasma concentrations of TRP, LNAAs, and TRP metabolites after correction for baseline values

Time (h)	TRP intake (mg)					LNAA intake (mg)					TRP/LNAA intake				
	1	2	3	4	6	1	2	3	4	6	1	2	3	4	6
Absorption															
TRP (mg)	0.90[1]	0.95[1]	0.90[1]	0.95[1]	0.93[1]	–0.49[1]	–0.19	–0.84[1]	–0.46[1]	–0.28	0.96[1]	0.97[1]	0.93[1]	0.93[1]	0.97[1]
LNAAs (mg)	0.52[1]	0.07	0.26	0.46	0.48	–0.26	–0.28	–0.20	0.17	0.36	0.56[1]	0.09	0.28	0.28	0.43
Portal vein															
TRP	0.78[1]	0.87[1]	0.91[1]	0.91[1]	0.12	–0.01	0.30	–0.05	–0.11	0.07	0.47[1]	0.55[1]	0.46[1]	0.58[1]	–0.01
LNAAs	0.54[1]	0.52[1]	0.31	0.25	–0.07	0.27	0.47[1]	0.51[1]	0.43	0.44	0.07	0.09	–0.41	–0.25	–0.45[1]
TRP/LNAA	0.80[1]	0.76[1]	0.85[1]	0.61[1]	0.74[1]	–0.04	–0.13	–0.29	–0.29	–0.11	0.55[1]	0.83[1]	0.64[1]	0.52[1]	0.75[1]
Serotonin	–0.44	–0.51[1]	–0.49[1]	–0.03	–0.46[1]	–0.27	–0.03	–0.38	0.23	–0.28	0.14	–0.49	0.19	–0.16	–0.07
Melatonin	0.04	0.84[1]	0.83[1]	0.50[1]	0.32	–0.10	0.31	–0.07	–0.18	0.07	–0.12	0.47[1]	0.49[1]	0.37	0.10
Kynurenine	0.38	0.69[1]	0.90[1]	0.35	0.68[1]	–0.17	0.41	–0.07	0.08	0.04	0.30	0.28	0.50[1]	0.02	0.53[1]
3HK	0.21	0.64[1]	0.85[1]	0.33	0.34	–0.05	0.36	–0.34	–0.12	0.02	0.04	0.35	0.71[1]	0.14	0.18
Left ventricle															
TRP	0.91[1]	0.93[1]	0.92[1]	0.93[1]	0.88[1]	0.05	0.36	–0.13	–0.14	–0.02	0.53[1]	0.51[1]	0.55[1]	0.62[1]	0.70[1]
LNAAs	0.74[1]	0.70[1]	0.58[1]	0.57[1]	0.10	0.32	0.55[1]	0.27	0.43	0.45[1]	0.15	0.12	–0.07	–0.10	–0.29
TRP/LNAA	0.81[1]	0.68[1]	0.84[1]	0.84[1]	0.12	–0.11	–0.15	–0.38	–0.19	–0.05	0.67[1]	0.79[1]	0.71[1]	0.62[1]	0.25
Serotonin	0.37	0.22	0.30	0.49[1]	0.32	–0.20	0.07	–0.35	–0.05	–0.14	0.36	0.13	0.33	0.40	0.27
Melatonin	0.39	0.73[1]	0.74[1]	0.65[1]	0.62[1]	0.16	0.34	0.14	0.19	0.23	–0.04	0.35	0.23	0.26	0.26
Kynurenine	0.43	0.69[1]	0.89[1]	0.34	0.60[1]	–0.08	0.12	–0.11	–0.09	0.21	0.19	0.37	0.52[1]	0.28	0.35
3HK	–0.48[1]	0.49[1]	0.52[1]	0.53[1]	0.57[1]	–0.06	0.23	–0.31	–0.15	0.17	–0.36	0.13	0.49[1]	0.32	0.37

Abbreviation: 3HK, 3-hydroxykynurenine.

Correlations (*r* values) determined with Spearman's correlations.

[1] *R* values were considered significant ($r \geq 0.5$ and $P \leq 0.05$).

TABLE 7

Correlations (*r* values) between absorption and plasma (portal vein and left ventricle) concentrations of tryptophan (TRP), LNAAs, and TRP metabolites (serotonin, melatonin, kynurenine, and 3HK) after correction for plasma baseline values

	True absorption		Portal vein concentrations (μmol/L)			Left ventricle concentrations (μmol/L)		
	TRP	LNAAs	TRP	LNAA	TRP/LNAA	TRP	LNAA	TRP/LNAA
True absorption								
TRP	N/A	0.53[1]	0.47[1]	0.03	0.19	0.67[1]	0.18	–0.01
LNAAs	0.53[1]	N/A	–0.08	–0.10	0.07	0.01	–0.09	–0.13
Portal vein								
TRP	0.47[1]	–0.08	N/A	0.61[1]	0.20	0.89[1]	0.66[1]	0.33
LNAAs	0.03	–0.10	0.61[1]	N/A	0.12	0.33	0.93[1]	0.16
TRP/LNAA	0.19	0.07	0.20	0.12	N/A	0.24	0.17	0.12
Serotonin	–0.22	–0.09	–0.32	–0.67[1]	0.03	–0.11	–0.63[1]	–0.02
Melatonin	0.27	0.09	0.21	0.00	0.12	0.35	0.05	0.12
Kynurenine	0.44	0.14	0.41	0.08	0.20	0.52[1]	0.14	0.14
3HK	0.31	0.13	0.28	0.10	0.15	0.31	0.10	–0.09
Left ventricle								
TRP	0.67[1]	0.01	0.89[1]	0.33	0.24	N/A	0.49[1]	0.30
LNAAs	0.18	–0.09	0.66[1]	0.93[1]	0.17	0.49[1]	N/A	0.18
TRP/LNAA	–0.01	–0.13	0.33	0.16	0.12	0.30	0.18	N/A
Serotonin	0.04	–0.03	0.11	0.15	0.09	0.09	0.12	0.01
Melatonin	0.46[1]	0.17	0.41	–0.02	0.05	0.54[1]	0.08	0.25
Kynurenine	0.49[1]	0.18	0.36	–0.02	0.16	0.53[1]	0.07	0.18
3HK	0.37	0.19	0.17	–0.07	0.08	0.30	–0.02	0.02

Abbreviations: 3HK, 3–hydroxykynurenine; LNAA, large neutral amino acid.
Correlations (*r* values) determined with Spearman's correlations.
[1] *R* values were considered significant ($r \geq 0.5$ and $P \leq 0.05$).

We observed that plasma kynurenine concentrations were several orders higher than those of melatonin. TRP metabolism through the kynurenine pathway is much greater than that through the serotonergic pathway [58,59], and as such, higher concentrations of the kynurenine pathway are expected. The TRP-dependent increase in kynurenine metabolite concentrations after increased absorption of TRP in this study is in agreement with the TRP dose-dependent plasma kynurenine concentrations in a previous pig study where animals were provided with food ad libitum with increasing TRP levels of between 0.14% and 0.35% TRP [53]. Increased availability of TRP after the AL and WPI diets likely caused an increased flux of TRP to the liver, where 3HK and kynurenine are produced [22]. However, appearance of plasma kynurenine and 3HK concentrations did not closely follow the pattern of absorption or plasma TRP appearance of the pigs fed AL or WPI meals as shown by the lack of correlation between absorption of TRP and plasma postprandial kynurenine and 3HK concentrations. Potentially TRP 2,3-dioxygenase, the rate-limiting enzyme of the hepatic kynurenine pathway [60], was a limiting factor in the flux of TRP through the hepatic kynurenine pathway.

This study did not find any meal effects on plasma serotonin concentrations. Almost all serotonin in blood is found circulating in platelets; only a very small amount circulates free [29]. Because platelets cannot synthesize serotonin from TRP [61], serotonin is thought to be synthesized in the gut, and released into blood where it is absorbed and sequestered in platelets. Hence, meal effects may have been found if serotonin was measured in platelets, but our data do not allow us to make any inferences on effects of TRP absorption on gut serotonin production.

In contrast to serotonin, meal effects were found on plasma melatonin concentrations, with highest concentrations after the AL meal. Although production of melatonin in the brain is controlled by lighting (for example, the day night cycle) [62]; more recently, melatonin has been found in other sites in the body, including the gut [63]. Several studies reported that the ingestion of meals containing proteins differing in their TRP contents elicits rapid, dose-related increases in TRP concentrations in blood (rat; human) [19,64]. This study adds that apart from plasma TRP concentrations alone, the AL meal led to a concomitant >10-fold increase in portal plasma melatonin concentrations at 3 and 4 h after the AL meal was ingested. The design of this study did not allow us to obtain gut tissue samples for direct measurement of melatonin concentrations. However, melatonin is a lipid-soluble molecule, and readily crosses cell membranes, once synthesized [62,65]. Hence, it is not unreasonable to suggest that the rise in plasma melatonin concentration after AL ingestion reflects increased melatonin production in gut tissue. The absence of a notable rise in portal plasma melatonin after ingestion of the WPI meal, despite a considerable though smaller rise in plasma TRP compared with the AL meal, suggests that this requires a large stimulation of gut melatonin synthesis to become reflected in portal blood melatonin concentrations. Although the findings overall are not definitive, the effect of ingesting AL on portal plasma TRP and melatonin levels is sufficiently interesting to warrant further study.

It should be noted that the tomato soup, used for the test meals, could have contained small amounts of melatonin [66]. However, the amount would have been small, and was consistent across the dietary treatments.

In conclusion, AL intake resulted in sustained elevated plasma TRP concentrations after a 12-h overnight fast. Plasma TRP, TRP/LNAA ratios, and TRP metabolites tended to increase along with increasing TRP from the dietary protein. In general, largest increases in plasma TRP concentrations were observed after the ingestion of protein with the highest TRP concentration (AL), as

a result of the largest amount of TRP absorbed. Plasma TRP, TRP/LNAA, and melatonin concentrations remained elevated for 4–6 h after administration of AL. Despite linear absorption patterns for TRP and LNAAs from the small intestine, postprandial plasma concentrations of TRP, LNAAs, and TRP metabolites did not follow this pattern, indicative of AA uptake and metabolism upon absorption. The findings of the study imply that, in adult humans, postprandial plasma TRP and TRP metabolites can be modified depending on the TRP content of the meal.

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Author contributions

The authors' contributions were as follows – PJM, CAM, SH, WCMN, JDF: were responsible for the planning of the pig study; CG, KK, LJM, KF, CAM: were involved in the laboratory analyses of the samples; CG, CAM: performed the statistical analyses; CG: prepared the first draft of the manuscript that was revised by CAM, LJM, KF, NCR, JDF, WCMN, PJM; and all authors: read and approved the final manuscript.

Conflict of interest

The authors disclose no conflict of interest.

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Animal welfare statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes.

Data availability

The raw data supporting the conclusions of this article can be made available by the authors, upon reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2024.07.010.

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