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Plasma Free Amino Acid Profiles Following an Acute Adrenaline Challenge in Iberian and Landrace Pigs: An Exploratory Study

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Simple Summary

The Iberian pig and modern lean breeds differ greatly in growth rate, body composition and metabolic characteristics. Understanding how these breeds respond to physiological stress signals may help characterize their distinct metabolic phenotypes. In this study, Iberian and Landrace pigs were subjected to an adrenaline challenge, a model used to simulate an acute stress response. Blood samples were collected repeatedly to evaluate plasma metabolites and free amino acid concentrations. We detected no statistically significant changes in plasma free amino acid concentrations over the 105 min after adrenaline injection. Across this period, Iberian pigs had higher average plasma tryptophan and tyrosine concentrations and lower average plasma creatinine concentrations than Landrace pigs. These differences remained statistically significant after accounting for multiple comparisons. These findings suggest distinct circulating metabolite and amino acid profiles in the two breeds. Because plasma concentrations reflect the net balance of several unmeasured processes, they do not identify differences in amino acid utilization, protein turnover, or tissue function.

Abstract

Pig breeding programmes that favour faster growth and greater lean tissue accretion have substantially reshaped the metabolic profiles of modern breeds. However, little is known about how pigs with contrasting genetic backgrounds respond to acute hormonal signals involved in nutrient metabolism. We compared how plasma metabolite and free amino acid concentrations changed in Iberian and Landrace pigs after a single dose of adrenaline. Ten barrows (initial body weight 25 ± 0.4 kg) were surgically fitted with carotid artery catheters for repeated blood sampling. One pig was subsequently excluded owing to catheter-related complications, leaving four Iberian and five Landrace barrows. All pigs received the same diet, which met Iberian protein requirements but was below those of Landrace pigs, and were fasted for 16 h before the adrenaline challenge. At approximately 50 kg body weight, pigs received a single intra-arterial injection of adrenaline (3 μg/kg body weight), and blood was sampled over the following 105 min. Plasma metabolites and free amino acids were analysed using automated colorimetric assays and HPLC, respectively. The primary analysis detected no significant time or breed × time effects for

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any outcome over the 105 min after injection. Plasma creatinine concentrations were lower in Iberian than in Landrace pigs both before injection and across the post-injection period. Across the post-injection period, Iberian pigs had higher plasma tryptophan and tyrosine concentrations and a higher tryptophan-to-large neutral amino acid ratio. No significant breed differences were detected in the summed amino acid pools. These findings identify breed-associated differences in selected circulating analytes under the feeding conditions studied. The breed associations observed here should be interpreted in light of dietary protein adequacy, age and farm of origin. The small sample size limits the precision of estimates of the acute response.

Keywords: catecholamines; amino acid metabolism; plasma amino acid profile; stress metabolism; Iberian pig; Landrace pig; metabolic adaptation

1. Introduction

Breeding for faster growth, more efficient feed use and greater lean tissue accretion has improved productivity in modern pig breeds. Selection for these traits may also have influenced the hormonal and metabolic processes that govern how pigs use nutrients and adapt to stress [1]. In particular, differences in hormonal regulation between genetically lean and obese pig breeds may influence the way animals mobilize and utilize nutrients under challenging physiological conditions. Understanding these differences is important not only for improving production efficiency, but also for evaluating the physiological resilience and welfare of animals raised under intensive production systems.

Among the hormones involved in metabolic regulation during stress, catecholamines play a central role. Adrenaline and noradrenaline are rapidly released following activation of the sympathoadrenal system and are key mediators of the acute stress response. These hormones prepare the organism for "fight-or-flight" situations by mobilizing energy reserves through glycogenolysis, lipolysis, and changes in glucose metabolism [2–4]. Catecholamine-mediated responses may differ between pig genotypes with contrasting body composition and selection history. We recently showed that adrenaline differentially affects carbohydrate and lipid metabolism in Iberian and Landrace pigs, with breed-specific glycolytic and lipolytic responses following adrenergic stimulation [5]. However, whether plasma free amino acid responses to adrenergic stimulation differ between these genotypes remains unknown. Studies in humans and rodents indicate that adrenaline can alter muscle protein turnover and circulating amino acid (AA) concentrations [6,7], but comparable evidence in pigs is scarce.

The absence of information on adrenergic effects on protein metabolism is particularly relevant when comparing pig genotypes with contrasting metabolic phenotypes. Native to the southwestern Iberian Peninsula, Iberian pigs tend to accumulate fat readily and have a limited capacity for lean tissue growth [8]. Compared with modern lean breeds, Iberian pigs exhibit high rates of muscle protein turnover with increased protein synthesis and degradation rates resulting in lower muscle protein accretion [9]. These characteristics have been associated with a "thrifty" metabolic phenotype that favors energy storage and adaptation to fluctuating nutritional conditions [10]. In contrast, lean commercial breeds such as Landrace have been intensively selected for rapid growth and lean tissue deposition. These contrasting characteristics make the two breeds a useful model for testing whether plasma amino acid responses to adrenergic stimulation differ between genotypes; however, they do not permit plasma concentrations to be interpreted as direct measures of protein metabolic fluxes.

Although Iberian and lean pig breeds differ in lipid metabolism, hormonal regulation and protein turnover [9,11], it remains unclear whether their circulating amino acid profiles differ following acute adrenergic stimulation. In particular, temporal changes in plasma free amino acid concentrations following an adrenaline challenge have not been characterized in these breeds. Plasma concentrations may provide insight into circulating profiles, although they do not directly measure nutrient mobilization, tissue uptake or protein turnover. Accordingly, the present study examined plasma free amino acids and selected biochemical variables associated with protein metabolism in the same Iberian and Landrace pigs previously studied by Lachica et al. [5], who reported their carbohydrate- and lipid-related metabolic responses to adrenaline. We addressed two related questions: first, whether plasma concentrations changed over time following adrenaline administration and whether these temporal profiles differed between breeds; and second, whether overall concentrations across the post-injection sampling period differed between breeds. An overall breed difference does not, by itself, indicate a difference in the response to adrenaline. A further aim was to identify breed-associated patterns in plasma amino acid profiles that may help characterize the physiology of Iberian pigs, including their higher energy expenditure compared with Landrace pigs [12]. We hypothesized that the two breeds would exhibit distinct temporal plasma metabolite and free amino acid profiles following adrenaline administration. We hypothesized that the two breeds would exhibit distinct temporal plasma metabolite and free amino acid profiles following adrenaline administration.

2. Materials and Methods

2.1. Animals and Experimental Design

The study involved conventionally bred Iberian and Landrace pigs (Sus scrofa domesticus) with no genetic modifications. The animals were clinically healthy at enrolment and had no previous experimental procedures. Ten barrows with a mean initial body weight of 25 ± 0.4 kg were used: five Iberian pigs of the Silvela strain from Sánchez Romero Carvajal (Jabugo S.A., Cádiz, Spain) and five Landrace pigs from Granja El Arenal (Córdoba, Spain). Iberian pigs were approximately 90 days old and Landrace pigs were 80 days old. Each pig occupied a 2 m^2 pen with a feeder, a drinker and environmental enrichment. The pens allowed visual contact between pigs and were located in a room maintained at 21 ± 1.5 °C, with 12 h of light and 12 h of darkness per day. Feed and water were freely available to the pigs throughout the experiment., except during the fasting periods associated with surgery and the sampling session. The same barley–soybean meal diet was offered to both breeds and was formulated to provide 126 g of crude protein and 14.3 MJ of metabolisable energy per kg. The diet was formulated to meet the estimated protein requirements of Iberian pigs recommended by FEDNA [13] for Iberian pigs, which have a lower lean growth potential than Landrace pigs. Consequently, dietary protein supply was considered suboptimal relative to the protein requirements of Landrace pigs. The ingredient and nutrient composition of the diet is presented in Supplementary Table S1. Feed intake was not recorded; therefore, actual protein and amino acid intakes could not be calculated.

Before the experiment, the pigs spent four weeks adapting to their pens. During this period, they were handled gently and petted daily to familiarize them with close human contact and reduce the risk of stress-induced catecholamine release during sampling. The Bioethical Committee of the Spanish Council for Scientific Research (CSIC, Spain) approved the study. All procedures complied with Spanish Royal Decree 1201/2005 and Council Directive 86/609/EEC of 24 November 1986 on the protection of animals used for experimental and other scientific purposes.

2.2. Surgical Procedures

A catheter was surgically placed in the carotid artery of each pig as described by Rodríguez-López et al. [14]. An incision was made along the jugular furrow to expose the carotid artery, which lay within a sheath alongside the vagus nerve and internal jugular vein, ventrolateral to the cervical vertebrae. The catheter was inserted into the carotid artery and advanced approximately 12 cm toward the aortic arch. The catheterisation allowed repeated blood sampling and intra-arterial administration while minimizing handling stress. Briefly, pigs were fasted overnight and premedicated with intramuscular ketamine (15 mg/kg body weight; Imalgene 1000, Merial, Barcelona, Spain) and azaperone (2 mg/kg body weight; Stresnil, Esteve, Barcelona, Spain). Isoflurane was administered via a face mask in oxygen flowing at 22–44 mL/kg body weight/min. The isoflurane concentration was 5% for induction and 0.5–2% for maintenance. N-butylhyoscine bromide + sodium metamizole (5 mL; Buscapina Compositum, Boehringer Ingelheim, Barcelona, Spain) was administered intramuscularly for analgesia and to prevent spasms. The catheter was advanced approximately 12 cm.

The free end of the catheter was externalized between the shoulders to prevent damage and facilitate access for sampling. For four days after surgery, pigs received intramuscular injections of the broad-spectrum antibiotic Duphapen Strep (Fort Dodge Vet S.A., Girona, Spain) at 5–10 mg/kg body weight per day. During recovery from anesthesia, each pig was kept in a separate cage and warmed with a heat lamp. One day after surgery, the pigs received Buscapina Compositum (Boehringer Ingelheim España S.A., Barcelona, Spain) for analgesia and to relieve spasms. The animals were fasted for 16 h but had free access to water. The postoperative day pigs received their pre-surgical daily feed allowance distributed in two meals. Feed and water intake were assessed qualitatively each day, and body temperature was also monitored daily. The approximate interval between surgery and sampling was two weeks. To keep the catheters patent, we flushed them daily with sterile 0.9% NaCl containing 250 IU of heparin per mL (PHARMACIA Spain S.A., Barcelona, Spain).

2.3. Adrenaline Challenge and Sampling

At the time of the adrenaline challenge, both breeds weighed approximately 50 kg. Supplementary Table S2 reports individual initial and final body weights, the number of days between weighings, and average daily gain. Average daily gain was calculated for each pig as the difference between final and initial body weights divided by the elapsed time between weighings. Their estimated ages were approximately 135 days for Iberian pigs and 122 days for Landrace pigs. Before the challenge, pigs were fasted overnight for approximately 16 h. At 10:00 h, each pig received a single intra-arterial dose of adrenaline (3 μg/kg body weight), prepared by diluting Adrenalina Braun (adrenaline bitartrate, 1 mg/mL; B. Braun Medical S.A., Rubí, Barcelona, Spain) in sterile saline. The catheter was immediately flushed with 5 mL of saline [15]. The final concentration of the adrenaline infusate was 50 μg/mL. At each sampling time, 5 mL of blood was drawn through the carotid catheter into a heparinized tube (Monovette VetMed; Sarstedt, Nümbrecht, Germany). Samples were taken 15 min before injection and at 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90 and 105 min after injection. Approximately 1 mL of the catheter content was discarded before sampling to clear dead space, and the catheter was flushed afterwards with heparinized saline. The −15-min sample was used as the baseline measurement, and the 0-min sample was collected immediately after injection. After collection, the blood samples remained on ice until they were centrifuged at 1820× *g* for 30 min at 4 °C using an Eppendorf 5810 R centrifuge (Hamburg, Germany). Plasma was deproteinized before storage by adding 20% trichloroacetic (27242; Riedel-de Haën, Seelze, Germany) acid plus norleucine (N1398; Sigma-Aldrich, St. Louis, MO, USA) at a plasma-to-reagent volume ratio of 1:1.

After centrifugation conditions or other separation step, the supernatant was aliquoted and stored at −20 °C for approximately 12 months. Amino acids were quantified in the deproteinized fraction without protein hydrolysis and is reported as plasma free amino acids. Each aliquot was thawed only once for analysis. One Iberian barrow was excluded because its catheter lost patency, preventing blood collection.

2.4. Chemical Analyses

Plasma albumin, creatinine and urea were analysed by colorimetry at the Instrumental Techniques Laboratory of the University of León (León, Spain) using an automated BA 400 analyser (Biosystems S.A., Barcelona, Spain) to quantify. All samples were analysed in a single run. The intra-assay coefficients of variation were 3.9% for albumin, 4.4% for creatinine and 4.0% for urea.

Plasma free amino acids were derivatised with phenylisothiocyanate (26922; Thermo Scientific, Rockford, IL, USA) before separation on a reversed-phase Waters Pico-Tag column (300 mm × 3.9 mm i.d.) using a Waters Alliance 2695 HPLC system (Milford, MA, USA). The analysis followed the Pico-Tag protocol for physiological amino acids [16], with matrix effects corrected using standard-addition recovery experiments [17]. Analyte-specific coefficient of variation and limits of detection were not determined for the porcine plasma samples analysed in this study. Plasma amino acid concentrations were quantified from calibration curves using the ratio of each analyte's peak area to that of the internal standard, norleucine. Chromatographic data from the Alliance HPLC system were processed with Waters Millennium 32 Chromatography Software (version 3.05.01). The chemical laboratory at EEZ-CSIC performed the amino acid analyses. Samples were blinded during laboratory analysis.

2.5. Statistical Analysis

Sample size was determined by animal availability rather than a formal a priori power calculation. Ten barrows were initially enrolled, comprising five Iberian and five Landrace pigs. One Iberian pig was excluded because catheter failure prevented blood sampling, leaving four Iberian and five Landrace pigs for analysis. The individual pig was considered the experimental unit. Baseline concentrations were measured in samples collected at −15 min. The sample obtained at 0 min was collected immediately after adrenaline injection and was treated as the first post-injection measurement. Baseline concentrations were evaluated separately using one-way analysis of variance with breed as the fixed factor and one record per animal. No data were excluded from the analysis.

Post-injection concentrations were analysed separately for each of the 20 individual free amino acids and three biochemical variables by linear mixed models for repeated measures using IBM SPSS Statistics (version 32.0.0.0 (134); IBM Corp., New York, NY, USA). Breed (Landrace or Iberian), sampling time (0, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90 and 105 min) and their interaction were included as fixed effects. Time was treated as a categorical factor. Repeated observations were specified within pig (SUBJECT = animal) using a spatial-power [SP(POW)] residual covariance structure and the actual unequal sampling intervals, represented in five-minute units. Restricted maximum likelihood (REML) estimation and the Kenward–Roger denominator degrees-of-freedom approximation were used. The residual covariance specification represented the within-pig dependence. An additional random pig intercept was not fitted. The primary longitudinal analysis of between-breed concentration profiles was performed without baseline adjustment. The analysis of individual amino acids was exploratory. Table 1 reports unadjusted p values for breed, time and breed × time. For the breed effect, Benjamini–Hochberg FDR-adjusted q values were calculated separately for the 20 individual amino acids, three biochemical variables and six derived pools or indices. The essential amino acids pool was calculated

as the sum of valine, tryptophan, threonine, phenylalanine, methionine, lysine, leucine, isoleucine, histidine and conditionally essential arginine. The non-essential amino acids pool was calculated as the sum of tyrosine, serine, proline, glycine, glutamic acid, glutamine, cysteine, aspartic acid, asparagine and alanine. Total amino acids pool was calculated as the sum of the essential amino acids pool, including arginine, and the non-essential amino acids pool. The branched-chain amino acids pool was calculated as the sum of leucine, isoleucine and valine. The large neutral amino acids pool was calculated as the sum of valine, leucine, isoleucine, phenylalanine and tyrosine. Pools were calculated separately for each animal and sampling time. A pool was considered missing when any constituent AA measurement was unavailable; no imputation was performed. Baseline values of the derived amino acid pools and indices were analysed by one-way ANOVA. Post-injection values were analysed using the repeated-measures linear mixed models described above. A spatial-power covariance structure was used except for essential AA, non-essential AA, total proteinogenic AA and urea, for which compound symmetry was used because the spatial-power fits produced singular-Hessian warnings. The SPSS syntax is provided as supplementary material. As a sensitivity analysis, mixed models including a random pig intercept were fitted for tryptophan, tyrosine, creatinine, TRP/LNAA and total amino acids, retaining the original fixed effects, REML estimation and Kenward–Roger degrees of freedom. Residual covariance was modelled using spatial power or identity when convergence failed. Breed differences were also assessed using individual pig means (0–105 min) and two-sided exact permutation tests. These exploratory analyses did not alter the FDR adjustment of the primary models.

Table 1. Post-challenge plasma metabolite and free amino acid concentrations and derived amino acid indices in Landrace (n = 5) and Iberian (n = 4) barrows over 105 min following intra-arterial adrenaline administration.

Analyte	Landrace LSMean ± SE	Iberian LSMean ± SE	*p* Breed	q Breed	*p* Time	*p* Breed × Time
Essential amino acids						
Essential AA	1071.4 ± 149.5	1433.0 ± 167.2	0.151	0.203	0.485	0.904
Valine	244.4 ± 42.8	327.8 ± 47.9	0.232	0.397	0.628	0.694
Tryptophan	16.8 ± 2.8	49.5 ± 3.2	<0.001	<0.001	0.603	0.334
Threonine	226.2 ± 60.2	259.4 ± 67.3	0.721	0.801	0.589	0.832
Phenylalanine	18.0 ± 4.2	23.3 ± 4.7	0.426	0.609	0.713	0.751
Methionine	18.7 ± 4.2	23.2 ± 4.7	0.491	0.654	0.759	0.879
Lysine	85.2 ± 25.1	147.9 ± 28.1	0.132	0.294	0.300	0.546
Leucine	149.7 ± 24.6	191.1 ± 27.5	0.300	0.461	0.636	0.614
Isoleucine	126.3 ± 15.3	175.5 ± 17.1	0.048	0.160	0.414	0.993
Histidine	46.4 ± 8.9	54.6 ± 10.0	0.562	0.702	0.524	0.403
Arginine	139.8 ± 12.7	180.7 ± 14.2	0.046	0.160	0.929	0.987
Non-essential amino acids						
Non-essential AA	1361.7 ± 139.7	1633.1 ± 156.2	0.236	0.236	0.805	0.596
Tyrosine	35.9 ± 4.3	63.0 ± 4.8	<0.001	0.007	0.807	0.896
Serine	101.7 ± 13.1	140.3 ± 14.6	0.079	0.226	0.363	0.502
Proline	133.6 ± 21.6	173.9 ± 24.1	0.238	0.397	0.677	0.324
Glycine	361.0 ± 52.2	369.1 ± 58.4	0.919	0.919	0.471	0.423
Glutamate	242.6 ± 32.9	310.5 ± 36.8	0.192	0.384	0.899	0.457
Glutamine	229.5 ± 21.3	216.0 ± 23.9	0.679	0.799	0.490	0.215
Cysteine	5.3 ± 3.8	18.2 ± 4.2	0.043	0.160	0.532	0.149
Aspartate	24.5 ± 4.6	39.3 ± 5.1	0.039	0.160	0.210	0.134
Asparagine	30.7 ± 2.1	30.4 ± 2.4	0.916	0.919	0.445	0.571

Alanine	196.9 ± 29.7	272.5 ± 33.2	0.124	0.294	0.357	0.170
Total AA and derived indices						
Total proteinogenic AA	2433.1 ± 264.3	3066.1 ± 295.5	0.154	0.203	0.920	0.807
Branched chain AA	520.3 ± 77.0	694.3 ± 86.1	0.169	0.203	0.748	0.955
Large neutral AA	574.3 ± 74.0	780.6 ± 82.7	0.095	0.203	0.795	0.931
Tryptophan/large neutral AA	0.04 ± 0.01	0.07 ± 0.01	0.004	0.024	0.906	0.557
Biochemical variables						
Albumin	480.1 ± 20.1	494.8 ± 22.4	0.632	0.632	0.595	0.885
Creatinine	100.1 ± 3.7	57.6 ± 4.1	<0.001	<0.001	0.723	0.497
Urea	2.97 ± 0.28	2.58 ± 0.31	0.382	0.573	0.072	0.903

Values are estimated marginal means (least-squares means, LSMeans) ± standard errors (SE) from linear mixed models without baseline adjustment. The models included breed, time (12 post-injection levels, 0–105 min) and breed × time as fixed effects, with repeated measurements specified within pig. Concentrations are expressed in μmol/L, except urea (mmol/L); Tryptophan/large neutral AA is dimensionless. *p* values are unadjusted. Breed-effect q values are Benjamini–Hochberg false discovery rate-adjusted *p* values, calculated separately. AA, amino acids; essential AA include conditionally essential arginine; total proteinogenic AA = essential AA + non-essential AA; branched-chain AA (valine + leucine + isoleucine); large neutral AA (branched chain AA + phenylalanine + tyrosine). Post-injection models were fitted without baseline adjustment. A spatial-power covariance structure was used except for essential AA, non- essential AA, total proteinogenic AA and urea, for which compound symmetry was used because the spatial-power fits produced singular-Hessian warnings. Benjamini–Hochberg adjustment for the breed effect was applied separately across the 20 individual amino acids, three biochemical variables and six derived pools and indices, using the nominal *p* values reported in this table.

2.6. Availability of Data and Code

All data supporting the results of this study are available from the corresponding author upon request.

2.7. Disclosure of GenAI Use

Generative AI (OpenAI ChatGPT, model GPT-5) was used exclusively for linguistic refinement and formatting of the manuscript text in accordance with journal style. It was not used for data analysis, interpretation, or generation of scientific content.

3. Results

Pre-challenge plasma concentrations are presented in Table 2. At −15 min, Iberian pigs had higher baseline plasma tryptophan concentrations than Landrace pigs (45.49 ± 15.96 vs. 17.82 ± 10.79 μmol/L, respectively; nominal $p = 0.017$). A difference was also observed for aspartate (37.43 ± 5.20 vs. 18.53 ± 7.11 μmol/L; $p = 0.003$), glutamate (335.38 ± 44.20 vs. 222.49 ± 71.04 μmol/L; $p = 0.028$) and serine (164.02 ± 42.38 vs. 107.41 ± 25.96 μmol/L; $p = 0.042$). None of these individual amino-acid baseline contrasts remained significant after FDR correction (q = 0.171 for Tryptophan, 0.061 for ASP, 0.188 for GLU and 0.210 for SER). Baseline creatinine was lower in Iberian than Landrace pigs (53.26 ± 7.30 vs. 95.12 ± 9.57 μmol/L; $p = 0.000178$, q = 0.000535 for the three biochemical variables).

Across the post-injection period, Iberian pigs had higher estimated marginal mean concentrations of tryptophan (49.54 ± 3.16 vs. 16.80 ± 2.83 μmol/L; $p < 0.001$; q < 0.001) and tyrosine (63.04 ± 4.82 vs. 35.94 ± 4.31 μmol/L; $p = 0.001$; q = 0.007) than Landrace pigs (Table 2). The Tryptophan/large neutral AA ratio was also higher in Iberian pigs (0.07 ± 0.01 vs. 0.04 ± 0.01; $p = 0.004$; q = 0.024), whereas creatinine was lower (57.64 ± 4.13 vs. 100.05 ± 3.70 μmol/L; $p < 0.001$; q < 0.001). Nominal breed differences in isoleucine, arginine, cysteine

and aspartate did not remain significant after FDR adjustment (all q = 0.160). No significant overall breed differences were detected for essential AA, non-essential AA, total proteinogenic AA, branched chain AA, large neutral AA, albumin or urea. In targeted sensitivity models with a random pig intercept, nominal breed differences remained for tryptophan (p = 0.0045; denominator df = 6.98), tyrosine (p = 0.0345; df = 7.00) and creatinine (p = 0.0007; df = 7.00), but not for TRP/LNAA (p = 0.0757) or total amino acids (p = 0.1544). In a separate animal-level analysis, exact permutation p values based on one post-injection mean per pig were 0.0079 for each of tryptophan, tyrosine and creatinine, 0.0952 for TRP/LNAA and 0.1508 for total amino acids.

No significant time or breed × time effects were detected for any outcome over the 0–105-min post-injection period (all nominal $p \geq 0.072$ and $p \geq 0.134$, respectively). Thus, the overall breed differences were not accompanied by detectable differences in temporal profiles following adrenaline administration. In a supplementary mixed model that included the −15 min pre-injection baseline as a time level, we compared each of the 12 post-injection time points with baseline within each breed. Among the interpretable contrasts, none reached statistical significance after Bonferroni adjustment. Supplementary Table S3 reports the estimated differences and Bonferroni-adjusted 95% confidence intervals. The concentrations of plasma metabolites, selected amino acids and grouped amino acids through time are shown in Figures 1–3, respectively.

Table 2. Pre-challenge plasma free amino acid and biochemical concentrations in Landrace (n = 5) and Iberian (n = 4) pigs.

Analyte	Landrace	Iberian	p (ANOVA)	q (FDR)
Essential amino acids				
Essential AA	1153.3 ± 214.6	1324.1 ± 467.0	0.486	0.486
Valine	256.5 ± 103.6	309.2 ± 93.8	0.456	0.653
Tryptophan	17.8 ± 10.8	45.5 ± 16.0	0.017	0.171
Threonine	213.5 ± 73.0	232.0 ± 172.2	0.833	0.875
Phenylalanine	20.1 ± 11.9	26.6 ± 17.1	0.519	0.653
Methionine	20.6 ± 10.7	14.8 ± 4.7	0.357	0.630
Lysine	112.9 ± 26.3	101.9 ± 41.4	0.638	0.751
Leucine	156.1 ± 47.1	199.5 ± 50.5	0.225	0.500
Isoleucine	117.1 ± 35.9	198.1 ± 87.8	0.099	0.284
Histidine	50.9 ± 9.5	51.8 ± 7.1	0.875	0.875
Arginine	188.0 ± 78.6	144.8 ± 51.6	0.378	0.630
Non-essential amino acids				
Non-essential AA	1371.9 ± 218.6	1746.4 ± 250.1	0.047	0.250
Tyrosine	34.2 ± 19.2	68.5 ± 32.2	0.085	0.284
Serine	107.4 ± 26.0	164.0 ± 42.4	0.042	0.210
Proline	130.5 ± 27.7	169.4 ± 73.0	0.303	0.605
Glycine	386.9 ± 50.3	373.4 ± 162.4	0.863	0.875
Glutamate	222.5 ± 71.0	335.4 ± 44.2	0.028	0.188
Glutamine	243.5 ± 38.2	285.3 ± 118.9	0.477	0.653
Cysteine[1]	4.5 ± 2.5	36.0 ± 51.5	0.206	0.500
Aspartate	18.5 ± 7.1	37.4 ± 5.2	0.003	0.061
Asparagine	25.5 ± 11.9	38.1 ± 5.9	0.098	0.284
Alanine	198.5 ± 62.2	238.8 ± 116.1	0.522	0.653
Total AA and derived indices				
Total proteinogenic AA	2525.1 ± 417.1	3070.5 ± 695.7	0.186	0.250
Branched chain AA	529.6 ± 177.1	706.7 ± 206.8	0.208	0.250
Large neutral AA	583.8 ± 157.8	801.9 ± 251.4	0.154	0.250

Tryptophan/large neutral AA	0.03 ± 0.02	0.06 ± 0.03	0.112	0.250
Biochemical variables				
Albumin	487.2 ± 73.9	494.6 ± 32.5	0.858	0.858
Creatinine	95.1 ± 9.6	53.3 ± 7.3	<0.001	0.001
Urea	2.94 ± 0.49	2.65 ± 0.99	0.573	0.858

Values are means ± SD from samples collected 15 min before adrenaline administration. Concentrations are expressed in µmol/L, except urea (mmol/L); Tryptophan/large neutral AA is dimensionless. All outcomes had complete data for five Landrace and four Iberian pigs. Baseline breed comparisons were assessed by one-way ANOVA, with pig as the experimental unit and one observation per pig. *p* values are unadjusted; q values are Benjamini–Hochberg false discovery rate (FDR)-adjusted *p* values, calculated separately for 20 individual amino acids, three biochemical variables and six derived pools/indices. AA, amino acids; essential AA include conditionally essential arginine (valine + tryptophan + threonine + phenylalanine + methionine + lysine + leucine + isoleucine + histidine + arginine); non-essential AA (tyrosine + serine + proline + glycine + glutamate + glutamine + cysteine + aspartate + asparagine + alanine). Total proteinogenic AA = essential AA + non-essential AA; branched chain AA, branched-chain AA (valine + leucine + isoleucine); large neutral AA, large neutral AA (branched chain AA + phenylalanine + tyrosine); Tryptophan/large neutral AA, tryptophan/large neutral AA. Pools were recalculated from individual-sample concentrations. [1] Cysteine results should be interpreted cautiously because the observed concentrations were highly variable and stability under the study storage conditions was not assessed.

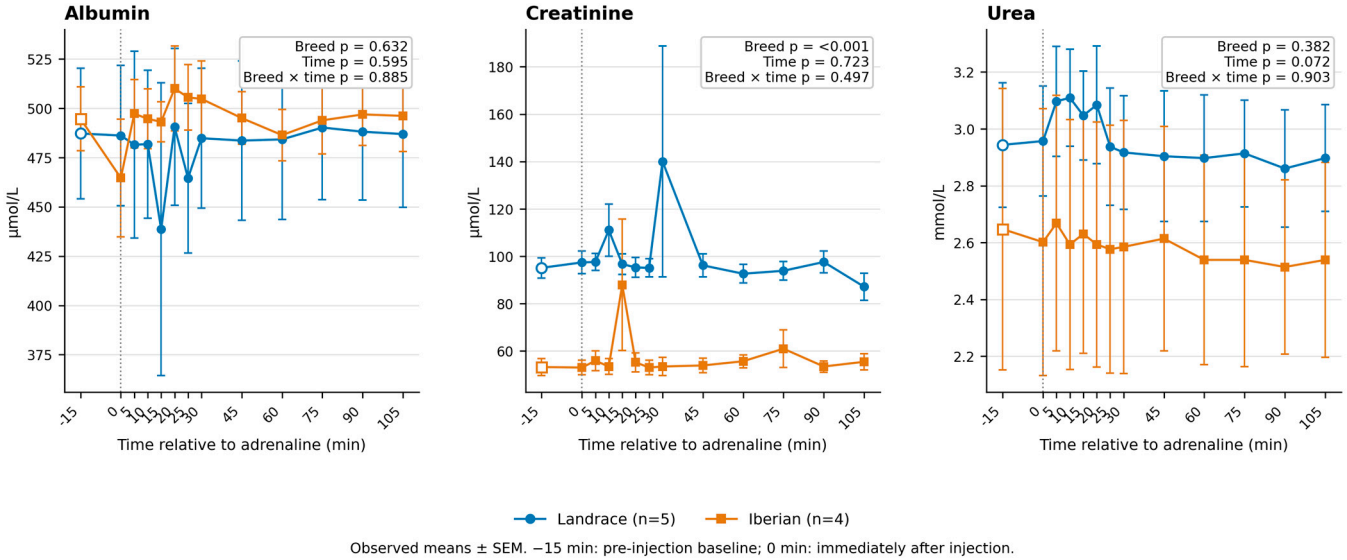


Figure 1. Longitudinal profiles of plasma biochemical parameters during the adrenaline challenge in Landrace and Iberian) pigs. Each time point per breed represents the average of 5 Landrace and 4 Iberian pigs, respectively. Panels show albumin, creatinine, and urea concentrations across sampling times. Data are presented as observed means ± SEM for each breed at each time point. The −15 min sample corresponds to the pre-injection baseline, whereas the 0 min sample was collected immediately after adrenaline administration. *p* values shown within each panel correspond to the global longitudinal mixed model for breed, time, and breed × time. Albumin and creatinine are expressed in µmol/L; urea is expressed in mmol/L. The horizontal dashed lines are gridlines included to facilitate reading values on the y-axis.

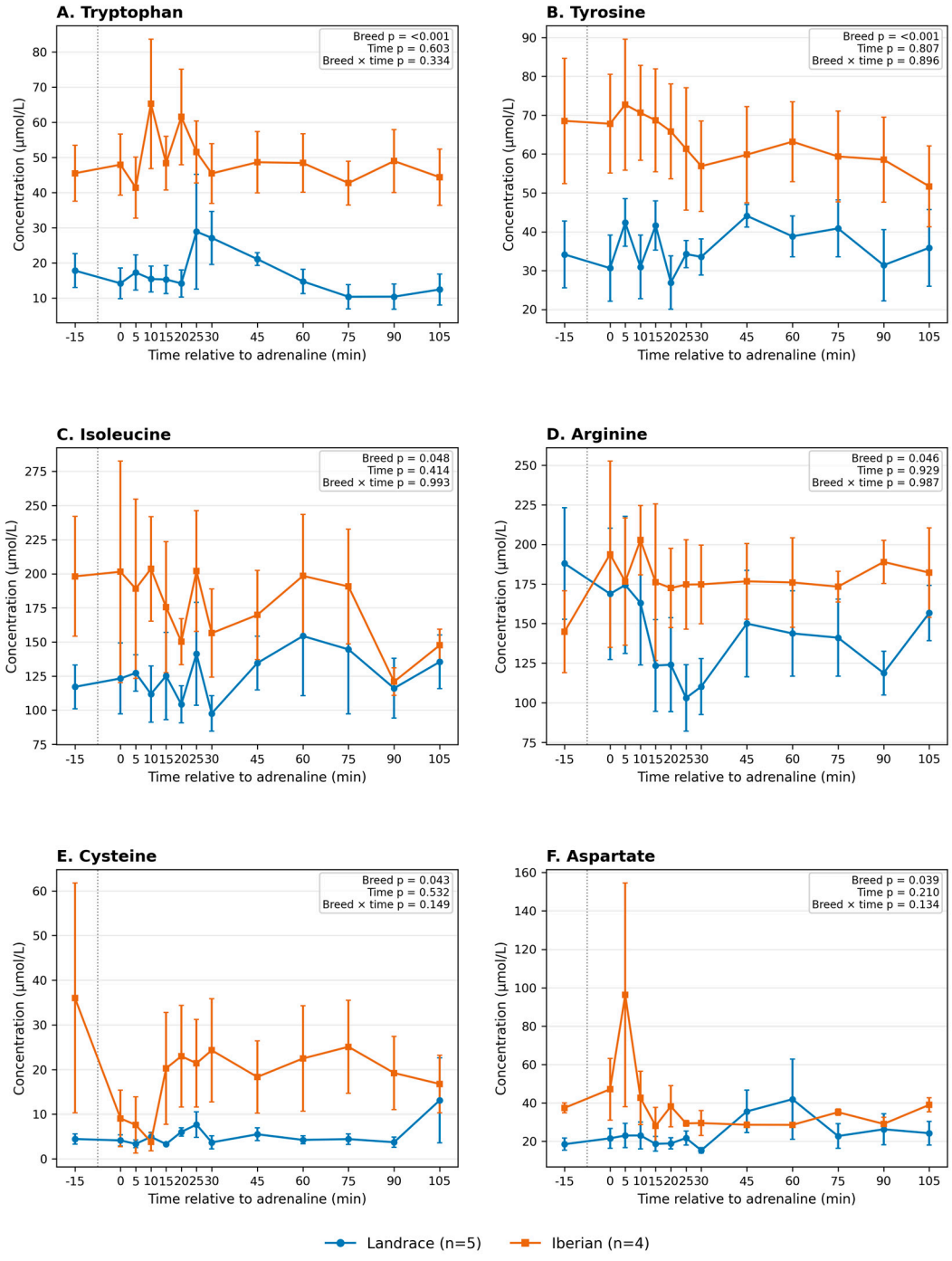


Figure 2. Longitudinal profiles of plasma free amino acids showing significant breed effects ($p < 0.05$) during the adrenaline challenge in Landrace and Iberian pigs but only tryptophan and tyrosine remained significant after FDR adjustment. Each time point per breed represents the average of 5 Landrace and 4 Iberian pigs, respectively. Panels show (**A**) tryptophan (TRP), (**B**) tyrosine (TYR), (**C**) isoleucine (ILE), (**D**) arginine (ARG), (**E**) cysteine (CYS), and (**F**) aspartate (ASP). Data are presented as observed means ± SEM at each sampling time. The −15 min sample corresponds to the pre-injection baseline, whereas the 0 min sample was collected immediately after adrenaline administration. The p values displayed within each panel correspond to the global longitudinal mixed model for breed, time, and breed × time interaction. For all six amino acids, the overall breed effect was significant ($p < 0.05$), whereas no significant overall time effect or breed × time interaction was detected. The horizontal dashed lines are gridlines included to facilitate reading values on the y-axis.

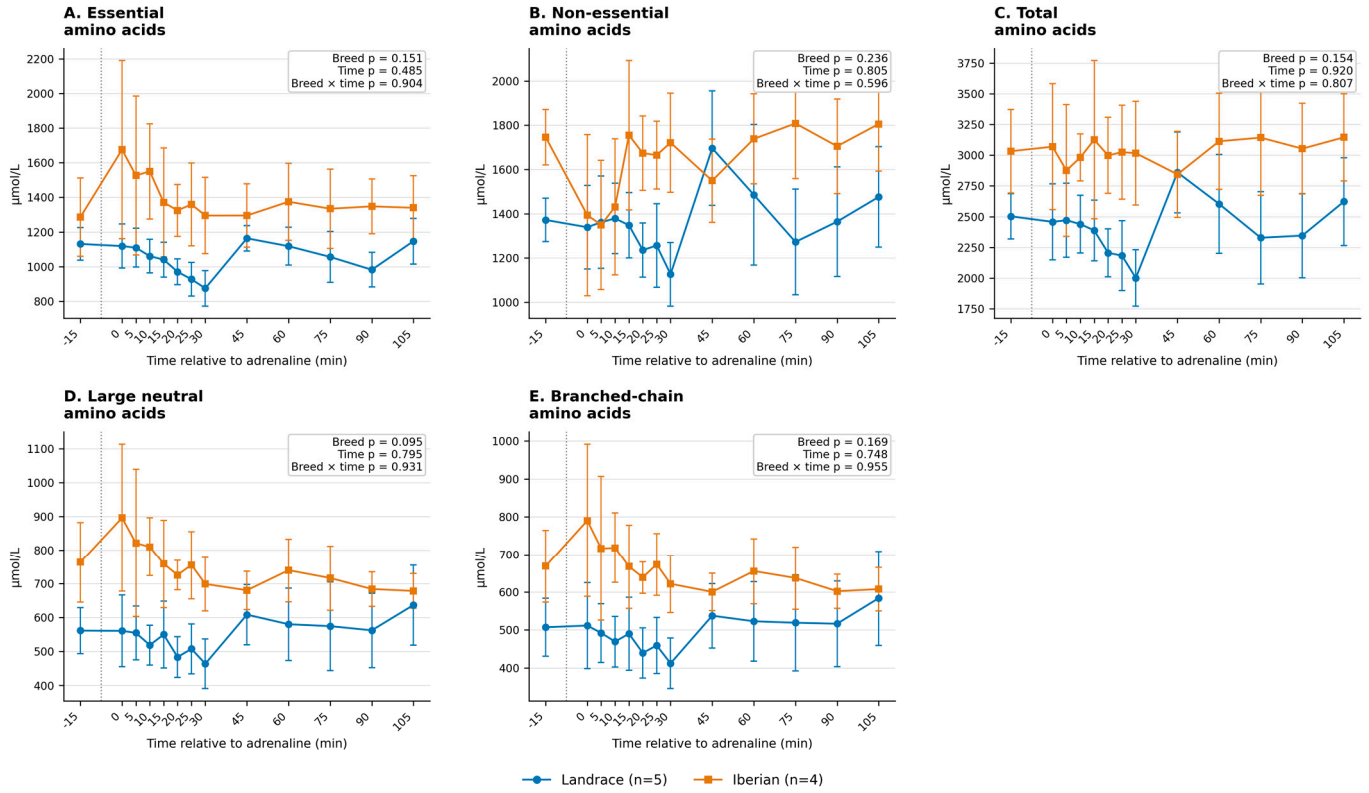


Figure 3. Longitudinal profiles of amino acid pools during the adrenaline challenge in Landrace and Iberian pigs. Panels show sum of essential amino acids, non-essential amino acids, total proteinogenic amino acids, large neutral amino acids and branched-chain amino acids. Data are presented as observed means ± SEM at each sampling time. Each time point per breed represents the average of 5 Landrace and 4 Iberian pigs, respectively. The −15 min sample corresponds to the pre-injection baseline, whereas the 0 min sample was collected immediately after adrenaline administration. The *p* values displayed in each panel correspond to the global longitudinal mixed model for breed (B), time (T) and breed × time interaction (B × T). No significant overall breed, time or breed × time effects were detected for these pooled variables. Concentrations are expressed in µmol/L. The horizontal dashed lines are gridlines included to facilitate reading values on the y-axis.

4. Discussion

Catecholamines, primarily adrenaline and noradrenaline, play a central role in the metabolic adaptations associated with the fight-or-flight response [18]. Adrenaline is released from the adrenal medulla and mobilizes metabolic substrates to meet increased physiological demands. Long-term adrenaline administration has been reported to increase N retention in pigs [19], although evidence on short-term effects is limited. In the companion study [5], the same Iberian and Landrace pigs received the same adrenaline challenge. That study reported acute changes in metabolites related to carbohydrate and lipid metabolism, including transient increases in plasma glucose and lactate, together with differences between breeds in their metabolic profiles [5]. A stimulation of lactate metabolism by exogenous adrenaline has also been demonstrated in humans [20]. The present manuscript extends the analysis of this experiment to plasma free amino acids and selected biochemical variables associated with protein metabolism. No significant temporal changes in plasma amino acid concentrations were detected in the present study. In contrast, overall concentrations of several AA differed between breeds across the post-injection sampling period. These findings address different questions: the temporal analysis examines the profiles following the challenge, whereas the overall breed comparison

describes differences in circulating concentrations under the conditions of the experiment. Plasma free amino acids concentrations reflect the balance of dietary appearance, tissue uptake and release, synthesis, oxidation, and clearance; they do not directly measure metabolic flux [21–23]. Consequently, the concentration differences observed here cannot establish differences in protein synthesis, proteolysis, protein deposition, or amino acids utilization. Isotope-tracer and transorgan balance techniques would be required to assess these processes directly [24]. Proposed explanations involving protein turnover, endocrine regulation, immune and antioxidant functions, or tryptophan metabolism should therefore be regarded as hypotheses for future investigation.

4.1. Acute Response to Adrenaline

Frequent serial sampling was used to characterize temporal patterns after the challenge, and the post-absorptive state minimized the immediate contribution of dietary amino acid absorption. Previous human and rodent studies reported lower concentrations of some plasma AA during adrenaline exposure and identified effects on muscle proteolysis using measurements beyond plasma concentrations [6,7,25,26]. These findings provide context, but their underlying mechanisms cannot be assumed to operate in pigs.

In the present study, no significant temporal changes in plasma free AA concentrations were detected over the 105-min sampling period following adrenaline administration. This finding does not establish the absence of a biological effect, as the limited number of animals may have reduced the ability to detect modest or variable responses. Differences in dose and exposure duration may also contribute to the contrast with human studies: the present experiment used a single dose of 3 μg/kg body weight, whereas studies reporting adrenaline-induced hypoaminoacidaemia used continuous infusions totaling approximately 9 μg/kg body weight over 3–5 h [6,27,28]. Because adrenaline is extracted by multiple tissues [29], exposure following a single injection may be shorter than during continuous infusion, although adrenaline kinetics were not measured here. These considerations provide possible explanations rather than demonstrated mechanisms. The absence of detectable temporal changes in circulating AA therefore does not exclude transient or tissue-level effects on protein turnover or AA flux. Although adrenaline inhibits insulin secretion under certain conditions [30,31], adrenaline infusion can increase plasma glucose and, to a lesser extent, insulin concentrations [27]. In the companion study, the challenge increased plasma glucose in these pigs [5]. In contrast, the present analysis detected no short-term temporal changes in circulating AA, urea, creatinine, or albumin. The lack of significant changes in plasma amino acid concentrations after the adrenaline challenge does not rule out changes in protein metabolism, as protein synthesis and breakdown were not measured directly. Species, dose, duration, and the sensitivity of the selected biomarkers may account for differences among studies.

The adrenaline challenge did not significantly change plasma urea, creatinine, or albumin over time. Because plasma urea concentration is influenced by urea production, distribution, and clearance, the absence of a detectable temporal change cannot be interpreted as unchanged nitrogen-use efficiency. Chronic adrenaline administration increased growth rate and N retention in 65-kg pigs [19], but the design and duration differed substantially from the present acute challenge. Heat stress studies likewise report inconsistent changes in blood urea and plasma AA [32,33], and involve hormonal and physiological responses beyond adrenaline. These comparisons therefore provide context but do not identify the mechanisms responsible for the present plasma profiles.

4.2. Breed-Associated Plasma Profile

The principal finding of the post-challenge breed comparison was the higher overall plasma concentrations of several individual AA in Iberian than in Landrace pigs. These

differences should be distinguished from differences in temporal responses following adrenaline administration, for which no significant breed × time effects were detected. Previously reported differences in energy expenditure [12], protein turnover and muscle fibre composition [9,34], and the description of a thrifty metabolic phenotype in Iberian pigs [10] provide physiological context for these profiles, although their contribution to the present findings remains unresolved.

The feeding strategy is central to interpreting the breed comparison. Both breeds received the same diet ad libitum, formulated to cover the estimated protein requirements of Iberian pigs but considered suboptimal relative to those of Landrace pigs [13]. Thus, the same dietary treatment did not provide equivalent protein adequacy for both breeds. Nitrogen retention efficiency has been reported to increase when dietary protein supply is suboptimal [35], raising the hypothesis that nutritional adaptation contributed to the plasma profiles of Landrace pigs. Overnight fasting reduced the immediate influence of a recent meal, but previous protein supply relative to requirements may still have affected the concentrations measured. Consequently, the observed differences should be interpreted within these feeding conditions, as nutritional adequacy cannot be separated from other breed-associated factors in the present design.

Comparative studies also indicate associations between circulating amino acid profiles and production phenotypes. Using a non-targeted ^{1}H-NMR metabolomic approach, Liaubet et al. [36] reported higher concentrations of several plasma AA in high-residual-feed-intake newborn piglets, whereas tryptophan concentrations were higher in low-residual-feed-intake piglets. These observations provide context for investigating individual AA patterns but do not establish a relationship between the present breed differences and feed efficiency. Similarly, differences in growth hormone secretion or sensitivity represent a hypothesis for future investigation, given previous breed comparisons [11]. The established involvement of arginine, glycine and proline in immune and antioxidant pathways [37,38] also raises the hypothesis that their availability or utilization differs between breeds. However, endocrine regulation, immune function and oxidative status were not assessed, and higher circulating concentrations do not demonstrate enhanced function in these pathways.

At the pre-injection baseline, mean plasma tryptophan concentration was 45.5 μmol/L in Iberian pigs and 17.8 μmol/L in Landrace pigs, a ratio of approximately 2.6. This descriptive difference was larger than the 48% difference reported by Palma-Granados et al. [39] in pigs at 25 kg body weight. The calculated dietary SID tryptophan concentration was below the FEDNA reference for commercial growing pigs (Supplementary Table S4), so dietary tryptophan supply may have contributed to the lower plasma concentration in Landrace pigs. Individual feed intake was not recorded, and the study design leaves the contributions of breed and dietary amino acid adequacy unresolved. Tryptophan is a precursor of serotonin, and its metabolism is linked to immune and inflammatory pathways [40]. Its competition with other large neutral AA for transport across the blood–brain barrier provides the rationale for examining the plasma tryptophan/large neutral AA ratio [41], which was also higher in Iberian pigs. Nevertheless, this ratio does not establish increased brain tryptophan uptake, serotonergic activity or reduced aggression, and behavioral associations reported in dietary or selection studies [42–44] cannot be inferred here. One hypothesis is that the breeds differ in tryptophan partitioning among the kynurenine, serotonergic and microbiota-derived indole pathways [45]. Investigating this hypothesis would require measurements of pathway-specific metabolites and inflammatory status; these were not assessed in the present experiment.

Plasma creatinine was more concentrated in Landrace pigs than in Iberian pigs. The greater lean mass generally associated with the Landrace breed [9] could help explain this

difference, although circulating creatinine also depends on its production, volume of distribution and renal clearance [46]. Because neither body composition nor creatinine kinetics was assessed in this study, the contribution of muscle mass could not be determined. Plasma urea concentrations were numerically lower in Iberian than in Landrace pigs both before the challenge and across the post-injection period, but neither breed comparison was statistically significant ($q = 0.858$ and 0.573, respectively; Tables 1 and 2). Although blood urea nitrogen has been associated with nitrogen-use efficiency under controlled nutritional conditions [47], plasma urea concentration reflects both production and clearance. These data therefore do not establish greater nitrogen retention or reduced amino acid catabolism in Iberian pigs. The lower urea synthesis previously reported in isolated Iberian hepatocytes [48] raises the question of whether urea production differs between breeds in vivo. Testing this hypothesis would require nitrogen balance measurements and direct estimates of urea production and clearance under defined feeding conditions.

Albumin is synthesized and exported by the liver; studies in rat hepatocytes estimate that it accounts for 12–18% of hepatic protein synthesis [49]. Its relatively long half-life and homeostatic regulation limit its sensitivity to short-term changes. Although isolated hepatocytes from Iberian pigs previously showed greater albumin synthesis than those from Landrace pigs, without an effect of the insulin/glucagon ratio [48], this in vitro observation cannot be inferred from the circulating albumin concentrations measured here.

4.3. Limitations and Implications

The experiment did not include a vehicle-treated group. We did not assess amino acid stability during approximately 12 months of storage at −20 °C. Deamidation of glutamine and asparagine and oxidation of cysteine may have affected their measured concentrations. Potential cysteine oxidation and high measurement variability warrant caution when interpreting cysteine concentrations and the amino acid pools that include it. The present study should be considered exploratory because it was not prospectively powered to detect differences in each individual amino acid outcome. The small sample size resulted in limited precision and may have reduced the ability to detect modest temporal or breed-by-time effects. Conversely, the number of outcomes examined increases the possibility of chance findings; therefore, individual associations require confirmation in adequately powered studies. Furthermore, the study evaluated only a single acute adrenaline dose over a 105 min period and therefore does not address responses to prolonged exposure.

Overall, this study identified breed-associated differences in circulating AA and metabolite concentrations under the experimental feeding conditions, while no significant temporal changes in plasma free AA were detected following adrenaline administration. They should not be interpreted as evidence of specific differences in nutrient utilization, protein turnover, stress resilience, immune function, or production efficiency without direct measurements of these processes.

5. Conclusions

Plasma free amino acid concentrations showed no significant time or breed × time effects during the 105 min after intra-arterial adrenaline administration. Across the post-injection period, Iberian pigs had higher mean plasma tryptophan and lower mean plasma creatinine concentrations than Landrace pigs. Previously reported increases in glucose and lactate in the same animals support the physiological activity of the challenge [5].

The small sample size limited the ability to detect modest or short-lived changes in plasma amino acid concentrations. Age, farm of origin, and dietary protein adequacy may have contributed to the observed breed-associated differences. The diet was formulated

to meet the estimated protein requirements of Iberian pigs and was considered suboptimal for Landrace pigs. Larger studies with direct measurements of amino acid flux are needed to clarify the metabolic basis of these findings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani16233781/s1. Longitudinal plasma concentrations of essential amino acids in Landrace and Iberian pigs. Panels show (A) valine, (B) tryptophan, (C) threonine, (D) phenylalanine, (E) methionine, (F) lysine, (G) leucine, (H) isoleucine, (I) histidine, (J) arginine.: Supplementary Figure S1.pdf. Longitudinal plasma concentrations of non-essential amino acids in Landrace and Iberian pigs. Panels show (A) tyrosine, (B) serine, (C) proline, (D) glycine, (E) glutamate, (F) glutamine, (G) cysteine, (H) aspartate, (I) asparagine, (J), alanine: Supplementary Figure S2.pdf. Composition (g/kg) and chemical analysis of the diet: Supplementary Table S1.pdf. Individual body weights and average daily gains of Iberian and Landrace pigs: Supplementary Table S2.pdf. Exploratory within-breed contrasts between each post-injection sampling time and the −15 min pre-injection baseline in Iberian and Landrace pigs: Supplementary Table S3.pdf. Calculated standardised ileal digestible amino acid content of the experimental diet and reference concentrations for growing pigs. Values are expressed in g/kg feed as fed: Supplementary Table_S4.pdf. Concise SPSS syntax, 29-outcome reanalysis and five model-based breed contrasts: Supplementary_Concise_SPSS_Syntax.pdf.

Author Contributions: Conceptualization, I.F.-F., R.N. and M.L.; methodology, I.F.-F., M.L. and A.R.; validation, I.F.-F., R.N., M.L. and C.G.-C.; formal analysis, C.G.-C., I.F.-F., A.R., J.M.R.-L., L.G.-V. and M.L.; investigation, I.F.-F., A.R., M.L., R.N., L.G.-V. and J.M.R.-L.; resources, I.F.-F., M.L. and R.N.; writing—original draft preparation, I.F.-F. and A.R.; writing—review and editing, I.F.-F., A.R., M.L., R.N., J.M.R.-L., L.G.-V. and C.G.-C.; visualization, C.G.-C., I.F.-F., M.L., R.N. and A.R.; supervision, I.F.-F. M.L. and R.N.; project administration, I.F.-F.; funding acquisition, I.F.-F. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The experiments were conducted in September 2008 in accordance with Royal Decree No. 1201/2005 (Spain). The animal study protocol was approved by the Ethics Committee of the Consejo Superior de Investigaciones Científicas, Madrid, Spain (protocol code: 21/03/2022/045; approval date: 21 March 2022).

Informed Consent Statement: This study did not involve humans, so an informed consent is not applicable.

Data Availability Statement: The raw data supporting the conclusions of this study will be made available by the authors upon responsible request.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

AA Amino Acids

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