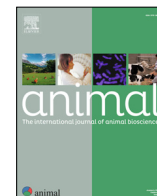




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Changes in carcass traits, proximate composition, amino acid profile and inflammatory markers in different muscles of pigs following glutamine supplementation



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ABSTRACT

Optimising nutritional strategies to enhance meat quality and animal welfare remains a major challenge in modern pig production. Early postweaning stress often impairs growth, muscle development, and immune responses, which can negatively affect carcass traits and pork quality. Among various nutritional interventions, amino acid supplementation has gained attention, with glutamine (Gln) considered particularly important due to its roles in metabolism, immunity, and muscle physiology. The present study aimed to investigate the effects of dietary Gln supplementation on the carcass traits, proximate composition, amino acid profile, inflammatory markers, and the most potent antioxidant in three different muscles (triceps brachii, longissimus lumborum - LL, and semimembranosus) in weaner gilts and barrows. The study involved 24 crossbred pigs, half barrows and half gilts, divided into two groups: one supplemented with 0.5% L-glutamine (0.5% Gln) and a control group. After a 42-day supplementation period just after weaning, pigs were slaughtered when weighing, on average, 123.77 kg, and muscle samples were collected for analysis. Results indicated that Gln supplementation significantly influenced carcass traits, including increased hot carcass weights and leanness, particularly notable in barrows. Proximate composition analysis revealed varied effects of Gln supplementation on protein and fat content across different muscles and pig sexes. Gln supplementation generally increased essential amino acid concentrations (especially in LL), with significant variations observed between sexes. Moreover, supplementation led to reduced concentrations of pro-inflammatory interleukin-1 β (IL-1 β) and tumour necrosis factor α (TNF- α) in certain muscles, indicating potential anti-inflammatory effects. However, there were no significant differences in instrumentally assessed muscle colour. These findings suggest that dietary supplementation with Gln can enhance pork's nutritional quality by improving protein composition and reducing muscle inflammatory markers. By enhancing essential amino acid concentrations and mitigating pro-inflammatory cytokines, Gln supplementation shows promise not only in improving meat quality but also in promoting animal health and welfare.

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Implications

Providing weaners with short-term glutamine supplementation can improve carcass traits, protein composition, and

reduce inflammatory markers at slaughter. This can potentially enhance meat quality and overall animal health, providing economic benefits to producers through higher-value products and improved growth efficiency. The findings support the hypothesis that early-life amino acid supplementation may provide long-term benefits, which could be applied in pig production to improve meat quality. These results benefit producers, meat processors, and consumers, offering insights into cost-effective

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strategies for improving pork production while supporting animal welfare.

Introduction

Current animal production systems are characterised by intensive fattening strategies aimed at achieving rapid growth and profitability. Consequently, optimising nutritional strategies to support animal health, improve carcass traits, and enhance meat quality has become a significant priority. Among these nutritional interventions, amino acid (AA) supplementation is particularly important because AAs are fundamental components required for efficient growth, robust immune responses, and optimal meat quality. A balanced AA profile in the diet is crucial for enhancing protein synthesis, reducing environmental stress, and improving overall production efficiency in pigs (Rhoads et al., 1991; Wu et al., 2011).

Glutamine (Gln) is recognised as a conditionally essential amino acid, especially important during periods of physiological stress, such as weaning or rapid growth phases. It serves as a key metabolic substrate and structural element, notably in rapidly proliferating cells such as enterocytes, lymphocytes, and skeletal muscle fibres. Gln is involved in various essential metabolic pathways, including nucleotide biosynthesis through purine and pyrimidine metabolism, and acts as a precursor to arginine, a critical amino acid for muscle and immune function (Morris et al., 2017; Yoo et al., 2020). Under physiological conditions, Gln constitutes more than half of the free AA pool circulating in the bloodstream, reflecting its substantial metabolic and physiological relevance (Demartini et al., 2020). While endogenous Gln synthesis primarily occurs in muscle tissue, significant amounts are also released from the lungs, brain, and adipose tissues (Cruzat et al., 2018). However, during periods of metabolic stress, such as weaning, illness, or intensive growth, the demand for Gln significantly increases, potentially leading to deficiency and compromising muscle growth and development (Ji et al., 2019).

The critical role of Gln supplementation in animal health and growth performance is increasingly documented. Previous research has established its benefits in supporting gut health, enhancing immunity, and reducing stress-induced muscle catabolism (Wu et al., 2011; Cruzat et al., 2018; Liu et al., 2024). Gln's immunomodulatory properties have been linked to its capacity to downregulate pro-inflammatory cytokines, such as IL-6, IL-1 β , and TNF- α , while simultaneously enhancing glutathione synthesis, an essential antioxidant protecting muscle tissue from oxidative damage (Cruzat, 2019; Chen et al., 2022; Liu et al., 2024). Furthermore, Gln has been shown to activate crucial anabolic pathways such as mTOR signalling, which activation not only promotes protein and lipid synthesis in muscle tissue but also plays a critical role in regulating feed intake, feed efficiency, and overall growth performance. These effects are particularly important in pig production, where improved nutrient utilisation translates directly into better productivity and economic outcomes (Kang et al., 2017; Yoshimura and Nomura, 2022).

Studies specifically evaluating Gln supplementation in pig nutrition have revealed promising results regarding gut integrity, growth performance, and health (Wu et al., 1996; Wang et al., 2008, 2022). For instance, dietary Gln supplementation in young piglets was demonstrated to enhance gut barrier function by promoting ATP synthesis through replenishing the Krebs cycle intermediates, significantly improving growth rates and feed efficiency during critical developmental windows (Wang et al., 2022). Most previous studies investigating Gln supplementation in pigs focused either on prenatal exposure or on the grower-finisher phase, reporting variable effects on carcass traits, intramuscular fat (IMF), and meat protein content (Hu et al., 2017; Vázquez-Gómez et al., 2021; Zheng et al., 2025).

However, data regarding its impact on key meat quality attributes such as pH, colour, or PSE incidence remain limited and inconclusive. Previous studies focused primarily on Gln supplementation during the growing-fattening phase, demonstrating its ability to modulate muscle fibre characteristics and improve antioxidant capacity and colour stability (Zhuang et al., 2018), or explored physiological determinants of meat colour variability without direct reference to Gln (Stufft et al., 2017). These parameters are closely associated with preslaughter stress, and given Gln's known ability to modulate stress and immune responses, its potential to improve meat quality deserves further investigation. Several studies suggest that Gln supplementation may mitigate the detrimental effects of preslaughter stress on pork quality. For instance, Ma et al. (2020) demonstrated that Gln reduced display and cook losses, lipid peroxidation, and discoloration in pigs exposed to heat and transport stress during weaning and finishing. These findings indicate that, beyond its metabolic roles, Gln may enhance resilience to acute stress, an important factor in the development of pale, soft, exudative-like conditions.

To our knowledge, no studies have evaluated the early post-weaning supplementation of Gln in relation to both carcass value and detailed meat quality outcomes. However, despite clear evidence supporting short-term benefits on gut health and growth performance in piglets, there remains a lack of comprehensive understanding regarding the long-term impacts of early-life Gln supplementation on carcass characteristics, muscle composition, and meat quality at slaughter.

Meat quality is intricately linked to its chemical composition, particularly protein and IMF content, and is influenced by various factors, including breed, diet, and sex of the animal (Alonso et al., 2009; Hu et al., 2017; Yuan et al., 2023). Previous research indicates significant variations between barrows and gilts regarding protein deposition and IMF content, which consequently affect carcass traits and consumer acceptability (Alonso et al., 2009; Yuan et al., 2023). Dietary strategies targeting enhanced protein deposition and optimal fat distribution are thus essential for improving meat nutritional value and sensory attributes.

Given these considerations, we hypothesised that dietary supplementation with Gln during the critical weaning period could improve growth performance, modulate muscle composition, and reduce inflammatory responses in pigs, ultimately enhancing meat quality and its nutritional profile at slaughter. Therefore, the present study aimed to evaluate the effects of short-term dietary Gln supplementation on carcass traits, proximate muscle composition, AA profile, and inflammatory markers in different muscle tissues (triceps brachii as a forelimb muscle with a relatively higher oxidative capacity, longissimus lumborum as a reference muscle commonly used in meat quality and nutritional studies due to its standardised anatomical location and intermediate fibre composition, and semimembranosus as a predominantly glycolytic hindlimb muscle) of barrows and gilts. To our knowledge, this is the first study that comprehensively investigates the impacts of early-life Gln supplementation on multiple muscle types in pigs, providing valuable insights for nutritional strategies aimed at optimising meat quality and animal welfare in intensive pig production systems. To test this, weaners were fed diets supplemented with two distinct levels of Gln for 42 days. Carcass characteristics, nutritional composition (including the AAs profile), inflammatory markers (cytokines), and non-enzymatic antioxidant glutathione levels were evaluated.

Material and methods

Study design

The present study describes an experiment designed to evaluate the effects of Gln supplementation on carcass traits, nutritional

composition, and inflammatory markers in weaner pigs. A completely randomised design was employed with two experimental factors: dietary treatment (control vs. 0.5% Gln) and sex (gilts vs. barrows).

The selection of a 0.5% Gln supplementation level was based on mechanistic insights and empirical evidence from previous studies that demonstrated significant metabolic benefits without adverse effects (Cabrera et al., 2013; He et al., 2016; Lee et al., 2003). This dosage is further justified by its role in enhancing gut integrity, supporting immune function, and promoting overall growth performance in piglets (Di Giancamillo et al., 2003; Teixeira et al., 2014). Consequently, it is expected to yield measurable improvements in carcass characteristics, muscle composition, inflammatory markers, and antioxidant status – parameters central to our hypothesis and experimental approach.

The 42-day supplementation period was chosen based on prior studies indicating that the weaner phase is critical for capturing changes in growth performance, muscle development, and metabolic adaptations (Smith et al., 2008; Yuan et al., 2023). This time-frame provides an optimal window to assess the impact of Gln supplementation on the aforementioned parameters (Collins et al., 2017).

Pigs were ear-tagged at weaning (28 days of age) and randomly assigned to the control or 0.5% Gln treatment group within their respective sex groups, ensuring that each treatment group consisted of an equal number of six barrows and six gilts. Independent measurements were assured by housing each pig individually in separate cages. Since supplementation was applied at the animal level, the experimental unit was the individual pig. Blocking was not implemented in the study, as the randomisation procedure was sufficient to balance potential sources of variation across treatment groups.

The number of animals required for the study was determined prior to the experiment in consonance with the resource equation approach (Festing and Altman, 2002; Festing, 2006; Mead et al., 2012; Charan and Kantharia, 2013; Arifin and Zahiruddin, 2017). According to the above mentioned, the acceptable range of degrees of freedom for the error term in an ANOVA should be kept between 10 and 20. Assuming a factorial ANOVA with interactions, four to six animals in each group established an error term, providing sufficient replication to estimate residual variance. Although no formal a priori power calculation was conducted, the resource equation approach ensured the study was adequately powered to detect significant effects of dietary Gln supplementation on the measured outcomes.

Experimental animals

All procedures were carried out in compliance with EU legislation (Directive 2010/63/EU regarding the protection of animals used for scientific purposes, as implemented in Poland by Legislative Decree 266/2015). Additionally, the research was conducted in line with the ARRIVE guidelines. Throughout the experimental period, a veterinarian regularly monitored the health of the pigs.

A total of 24 Polish Large White × Polish Landrace crossbred pigs (12 barrows and 12 gilts) from the same commercial line were used in this experiment. The piglets were weaned at 28 days of age and had an initial body weight of 12.97 ± 0.91 kg (mean $\pm$ SD). Within each sex, animals were randomly assigned to one of two dietary treatments: control or 0.5% L-glutamine supplementation, resulting in a 2×2 factorial design (factors: diet and sex). After randomisation, the initial body weight was compared between dietary groups using an unpaired two-tailed Student's *t*-test to verify proper randomisation. No significant differences were found within either sex ($P > 0.05$), confirming adequate group homogeneity. The ingredient composition and nutrient content of experimental diets (prestarter and starter) fed during the experimental

period (from 28 to 70 days of age) are presented in Tables 1 and 2, respectively. Amino acid content of experimental basal diets is shown in Table 3. Amino acids in the basal diets were determined using the official EU method for feed control (EC, 2009). According to this method, Gln is analysed together with Glu because Gln is converted to Glu during acid hydrolysis (Fontaine, 2003). The basal diet was formulated to meet the nutritional requirements for pigs of this age, according to National Research Council guidelines and Polish standards of pig nutrition (National Research Council, 2012; Grela and Skomiał, 2020). During the experimental period, the test group received 0.5% L-glutamine (Biomus, Lublin, Poland; purity $\geq 99.5\%$) added to the basal diet, while the control group was fed the basal diet without additional L-glutamine. The analysed Glu + Gln content in basal diets was 45.7 and 43.9 g/kg and in the experimental diets was 50.67 and 48.93 g/kg, in the prestarter and starter diets, respectively. From 71 to 160 days of age, both groups were fed the same diet without extra L-Gln.

All pigs were housed in individual pens (1×3 m), each equipped with a feeder and a water drinker, allowing *ad libitum* access to feed and water. Daily feed intake and weekly live weight gain were recorded, and feed conversion efficiency was calculated.

At 160 days of age (one day before slaughter), all pigs were transported 40 km to a commercial abattoir, where they were fasted overnight with free access to water (Dall Aaslyng and Barton-Gade, 2001). The following day, they were weighed, with a mean body weight of 123.8 kg (ranging from 111.3 to 134.0 kg). The animals were slaughtered by electrical stunning followed by exsanguination in accordance with standard commercial practices. Postmortem evaluations were conducted at the abattoir, including measurement of hot carcass weight, backfat thickness, and dorsal muscle thickness at the third to fourth last rib.

Carcass quality evaluation procedure and sample collection

The hot carcass was weighed and then corrected for the diaphragm, perirenal fat, and kidneys remaining *in situ*. Carcass composition was evaluated 35 min postmortem with an IM-03 opto-needle probe (ZTM-EXE, Poznań, Poland; needle diameter = 7 mm). The probe provided leanness (%), EUROP class, subcutaneous backfat thickness (F2), and longissimus thoracis muscle thickness (M2). Both F2 and M2 were taken at the same anatomical site, three-quarters of the distance along the last thoracic vertebra, 6 cm lateral to the dorsal mid-line, with the probe oriented parallel to the mid-line. The measurements followed the EU-authorised grading procedure for the classification of pig carcasses in Poland (EC, 2005), using the formula detailed in Part III of the Annex (EC, 2024).

Within 45 min after slaughter, muscle samples (~200 g each) of TB (central portion of the long head, midway between the scapular insertion and the olecranon), LL (taken between the first and fourth lumbar vertebrae), and SM (central part, approximately at the midpoint between the ischial tuberosity and the distal insertion near the stifle joint) of the left carcass half were collected, vacuum-packed, and stored at -20°C (Delgado-Suárez et al., 2016; Hu et al., 2017). In addition, 10 g samples of muscles, trimmed of superficial fat and connective tissue, were individually packed in cryotubes, frozen in liquid nitrogen, and then stored at -80°C for the subsequent analysis of cytokines and antioxidant markers, performed within 1 month of sample collection (Panicker et al., 2007). During the first 24 h postmortem, carcasses were stored in a ventilated cold room at $3\text{--}4^\circ\text{C}$.

pH and colour of longissimus lumborum muscle, and proximate chemical composition

The initial (pH 30 min) and final (pH 24 h) pH were measured at 30 min and 24 h postmortem, respectively, at the last rib level of

Table 1
Ingredients (%) of the pigs' feed mixtures (as-fed basis) fed during the experimental period (Jarosz et al., 2023).

Item	Phase 1 prestarter, 28–41 days of age, (13–20 kg BW)		Phase 2 starter, 42–70 days of age, (20–35 kg BW)	
	control ¹	0.5%Gln ²	control	0.5%Gln
L-Glutamine	–	0.5	–	0.5
Wheat	15.0	15.0	15.0	15.0
Barley	10.0	10.0	10.0	10.0
Triticale	10.0	10.0	15.0	15.0
Corn	27.62	27.12	25.36	24.86
Soybean meal	16.0	16.0	18.0	18.0
Wheat bran	–	–	10.0	10.0
Soybean oil	3.3	3.3	3.0	3.0
Skimmed milk powder	10.0	10.0	–	–
Dried whey	5.0	5.0	–	–
Sodium chloride	0.15	0.15	0.32	0.32
Phosphate 1-Ca	0.28	0.28	0.50	0.50
Limestone	1.20	1.20	1.50	1.50
Vitamin/mineral premix ³	0.5	0.5	0.5	0.5
L-Lysine	0.48	0.48	0.45	0.45
DL-Methionine	0.25	0.25	0.17	0.17
L-Threonine	0.22	0.22	0.20	0.20

¹ basal diet without glutamine supplementation
² group supplemented with 0.5% glutamine.
³ Vitamin/mineral premix provided per kg of diet: A 12 000 IU, D3 2 000 IU, E 40 IU, B1 2 mg, B12 30 mcg, B2 5 mg, B6 3 mg, K 2 mg, biotin 150 mcg, niacin 25.04 mg, folic acid 0.5 mg, pantothenic acid 13.8 mg, choline 120.96 mg, betaine 60 mg, Cu 100 mg, Fe 100 mg, I 1 mg, Mn 40 mg, Se 0.3 mg, Zn 120 mg, Ca 1.34 g, Cl 0.03 g; K 0.33 mg, Mg 0.15 g, Na 0.185 mg, S 0.11 g.

Table 2
Nutritional value of the pigs' basal diets (g/kg, as-fed basis) fed during the experimental period (Jarosz et al., 2023).

Variable	Phase 1 prestarter, 28–41 days of age (13–20 kg BW)	Phase 2 starter, 42–70 days of age (20–35 kg BW)
Metabolisable energy (MJ)	13.9	13.4
CP	180.0	170.0
DM	888.0	880.0
Crude fibre	24.0	35.0
Crude ash	52.0	53.2
Ether extract	52.8	52.3
Nitrogen-free extractives	579.2	569.5
Phosphorus (digestible)	3.0	2.7
Calcium	7.6	7.6
Lysine	14.2	12.2
Methionine + Cystine	8.5	7.4
Threonine	8.8	7.6
Tryptophan	2.5	2.4

LL. A meat pH meter (pH-Star, Matthäus, Pöttmes, Germany) was used, calibrated with standard buffer solutions of pH 4.0 and 7.0 (Tomaszewska et al., 2021).

Meat colour (CIELab scale: L*, a*, b*) was assessed 45 min post-mortem using a CR-400 chroma meter (Minolta, Osaka, Japan), equipped with a D65 illuminant, a 2° standard observer angle, an 8-mm aperture, and a pulsed xenon lamp as the light source (Dunshea et al., 2024). The apparatus was calibrated using a ceramic tile (CIE L* = 99.25, a* = -0.60, b* = 1.87), as recommended by the manufacturer. Measurements were performed in quadruplicate at different locations on unhomogenised, 2.5 cm-thick LL samples cut perpendicular to the muscle fibre's long axis. Prior to measurement, samples were exposed to air for 30 min at 4 °C to allow blooming (Brewer et al., 2001; Mathobela et al., 2025).

Homogenised muscle samples were analysed according to AOAC methods (2000) for moisture (oven drying method 934.01), IMF (ether extract method 920.39), and protein content (Kjeldahl system with a 6.25 × N conversion factor to crude protein, method 954.01). Analyses were performed at the Central Analytical Laboratory, University of Sciences in Lublin, Lublin, Poland, which is

Table 3
Amino acid content (g/kg) in basal diets fed to pigs during the experimental period.

Variable	Phase 1 prestarter, 28–41 days of age, (13–20 kg BW)	Phase 2 starter, 42–70 days of age, (20–35 kg BW)
Ala	7.23	6.61
Arg	10.3	9.31
Asp + Asn	14.4	12.4
Gln + Glu	45.7	43.9
Gly	7.52	6.95
His	4.55	4.17
Ile	6.20	5.78
Leu	12.4	11.6
Lys	12.7	10.4
Phe	8.70	8.11
Pro	14.2	13.6
Ser	8.52	7.92
Thr	8.28	7.25
Tyr	5.13	4.77
Val	7.63	7.29

Abbreviations: Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid (aspartate); Gln = glutamine; Glu = glutamic acid (glutamate); Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Tyr = tyrosine; Val = valine.

accredited according to ISO/IEC 17025:2017 by the Polish Accreditation Centre (certificate number AB 1375).

Amino acid analysis

Prior to determining the AA profile, feed and muscle samples were hydrolysed with 6 N HCl at 110 °C for 23 h under a nitrogen atmosphere. Sulphur-containing AAs (methionine and cystine) were oxidised to methionine sulphone and cysteic acid, respectively, using performic acid prior to hydrolysis. Analyses were carried out by ion-exchange chromatography on a INGOS AAA400 analyser (Ingos Corp., Prague, Czech Republic) equipped with an Ostion LG ANB ion-exchange resin column (370 × 3.7 mm). Separation took place at 76 °C with sodium citrate buffers (flow rate of 0.3 mL/min) containing 1.0 or 2.6 M sodium chloride. Quantification was performed with postcolumn ninhydrin derivatisation

(flow rate 0.2 ml/min) and spectrophotometric detection at $\lambda = 570$ nm (440 nm for proline) (EC, 2009). All analyses were performed in triplicate, and all sample replicates showed CV < 5%. During acid hydrolysis, Asn and Gln lose their amide residues, forming Asp and Glu, respectively. Therefore, they were analysed and reported as Asp + Asn and Glu + Gln. Results were expressed as g of each amino acid per gram of feed (on as-fed basis), and mg of each amino acid per gram of fresh muscle. Calibration of the amino acid analyser was performed using an amino acid standard (AAS18, Supelco, Merck KGaA, Darmstadt, Germany). Analyses were performed at the Central Analytical Laboratory, University of Sciences in Lublin, Lublin, Poland.

The chemical score (CS) of essential amino acids (EAA) and non-essential amino acids (NEAA) was calculated using the formula proposed by (Lorenzo et al., 2014). The CS index indicates how well (in percentages) a protein meets the requirements of each EAA relative to a reference pattern protein. The reference pattern protein for adults proposed by FAO/WHO/UNU (WHO et al., 2007) was used in this study.

Analysis of cytokines and antioxidant markers

The reduced glutathione (GSH) and cytokines (TNF- α , IL-1 β , IL-6) concentrations were measured in muscle samples. Briefly, 0.5 g of muscle was homogenised (Ultra-Turrax, IKA-Werke GmbH, Staufen, Germany) in 2 mL of ice-cold sucrose buffer (Graham, 2002) and then centrifuged at 4 °C for 10 min at 16 000 $\times$ g. The concentration of TNF- α , IL-1 β , and IL-6 in the supernatant was determined using commercial porcine-specific ELISA kits from BT-Lab (Shanghai, China) for TNF- α (#E0610Po), IL-1 β (#E0116Po), and IL-6 (#E0122Po). All values were normalised to the total protein content of each sample, measured with the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). GSH concentration was assessed using a commercial kit (#E-KC-030-M, Elabscience, Houston, Texas, USA). All assays were performed according to the manufacturers' protocols on a Benchmark Plus microplate spectrophotometer (Bio-Rad, Hercules, CA, USA). Each sample was assayed in triplicate, with intra-assay CV < 8% for all measurements.

Statistical analysis

A comprehensive statistical analysis was conducted to discover the changes in the studied variables under the influence of Gln supplementation. Data points identified as potential outliers were verified for biological plausibility and retained in the dataset unless clear evidence of measurement error was found. The assumptions of normal distribution and variance homogeneity were confirmed by the Shapiro-Wilk test and Levene's test, respectively. The variables were analysed using GLM. The analysis included two groups (control and 0.5% Gln), two sexes (barrows and gilts), and their interaction according to the following model (Eq. (1)):

$$y_{ijk} = \mu + G_i + S_j + (G \times S)_{ij} + \varepsilon_{ijk} \quad (1)$$

where: y_{ijk} – dependent variable; μ – mean value; G_i – group effect; S_j – sex effect; $(G \times S)_{ij}$ – group-sex interaction; ε_{ijk} – random effect. Posthoc comparisons were performed using Tukey's Honest Significant Difference (HSD) test. If the interaction term was insignificant ($P > 0.05$), the model was simplified by excluding the interaction, and the main effects of dietary treatment and sex were analysed independently. Model residuals were tested to validate the assumption of normality and homoscedasticity using QQ plots, the Shapiro-Wilk test, residual-fitted values plots and Bartlett's test. For

all analyses, $P < 0.05$ was considered statistically significant. Data were presented as least squares means with pooled SEM.

ANOVA modelling was performed in Statistica 13 (TIBCO Software Inc., Palo Alto, CA, USA); assumption diagnostics and posthoc comparisons were carried out in RStudio 2023.09.1 build494; (Posit Software, PBC, Boston, MA, USA); and all graphs were created with GraphPad Prism (ver. 10.5.0, GraphPad Software Inc., Boston, MA, USA).

Results

Performance, carcass and longissimus lumborum muscle traits

Feed conversion efficiency, daily feed intake and average daily gain were similar across all groups (Table 4). In contrast, Gln supplementation significantly increased slaughter weight ($P = 0.001$), with the results in barrows being better than the results in female gilts ($P = 0.002$). Accordingly, both hot carcass weight and carcass weight corrected for backfat were higher in the 0.5% Gln group, an effect particularly pronounced in barrows. Gln also altered fat thickness: F2 layer thickness decreased, whereas M2 increased. Leanness improved markedly following Gln supplementation ($P < 0.001$), shifting carcass classification from class E (control) to class S (0.5% Gln). Neither muscle pH at 30 min and 24 h nor colour parameters differed by sex or diet, and all values remained within the normal range, ruling out any pale, soft, and exudative (PSE) defect.

Proximate composition

Fig. 1 summarises the proximate composition of the analysed muscles. In LL, gilts fed 0.5% Gln showed a higher protein content than both barrows and control gilts ($P = 0.011$), while IMF was lowest in control gilts ($P = 0.023$). Moisture did not differ by sex or treatment. In TB, protein and moisture remained unchanged, while differences in IMF were observed only between control gilts and supplemented barrows ($P = 0.036$). In SM, Gln reduced protein content regardless of sex ($P = 0.013$), and an interaction ($P = 0.004$) caused a decrease in IMF only in Gln-treated gilts; moisture again showed no differences.

Amino acids

The supplementation of 0.5% Gln induced multiple, muscle-specific changes in AA profiles (Tables 5–7). In LL, Gln raised Thr ($P = 0.009$), Ser ($P = 0.014$), Ile ($P = 0.006$), Tyr ($P = 0.005$), Phe ($P = 0.015$), Lys ($P = 0.005$), Arg ($P = 0.030$) and Asp + Asn ($P = 0.041$). Sex alone affected Met ($P = 0.017$), higher in gilts, while interactions drove barrow-specific increases in Val ($P = 0.002$), Leu ($P = 0.014$), His ($P = 0.006$) and Glu + Gln ($P = 0.022$), and a gilt-only rise in Cys ($P < 0.001$). Total EAA and NEAA concentrations were higher with Gln ($P = 0.008$ in barrows), though the EAA/NEAA ratio (0.85) and %EAA (~45.9%) remained unchanged. Overall AA content peaked in Gln-supplemented barrows ($P < 0.001$), with a significant interaction effect ($P = 0.010$).

In TB, Gln had less impact. Group reduced Leu ($P < 0.001$) and Lys ($P = 0.008$), while total NEAA were higher in controls ($P = 0.002$) and the EAA/NEAA ratio and %EAA increased with Gln ($P = 0.008$ each). An interaction elevated Glu + Gln in barrows ($P = 0.016$) and Arg in control gilts ($P < 0.001$). In SM, gilts showed higher levels of most AAs (all $P < 0.05$ except His, Met, Cys), and an interaction increased Thr in Gln-fed gilts ($P = 0.037$). Females also had greater EAA and NEAA ($P < 0.001$) and, with Gln, a higher EAA/NEAA ratio and %EAA ($P = 0.015$).

Table 4

Performance and carcass traits of weaner pigs (gilts and barrows) in the control and experimental (0.5% Gln) groups.

Variable	control ¹		0.5%Gln ²		SEM	P-value		
	Barrows	Gilts	Barrows	Gilts		Group ³	Sex ⁴	Group × Sex ⁵
Number of animals	6	6	6	6				
Growth performance								
Slaughter weight (kg)	124.3	116.4	128.1	126.4	2.11	0.004	0.032	ns.
Carcass traits								
Hot carcass weight (kg)	96.9	90.6	100.3	99.1	1.47	0.001	0.025	ns.
Corrected carcass weight (kg)	94.8	89.0	98.6	97.0	1.73	0.002	0.038	ns.
F2 (mm)	17.1	14.1	10.8	9.9	1.00	<0.001	ns.	ns.
M2 (mm)	65.3	65.2	76.3	71.0	3.11	0.014	ns.	ns.
Leanness (%)	56.9	57.9	61.6	62.0	1.00	<0.001	ns.	ns.
Feed conversion efficiency (kg/kg)	0.73	0.72	0.72	0.72	0.031	ns.	ns.	ns.
Daily feed intake (kg)	0.72	0.68	0.69	0.63	0.553	ns.	ns.	ns.
Average daily gains (kg)	1.07	1.06	1.18	1.16	0.807	ns.	ns.	ns.
Longissimus lumborum traits								
pH 30 min ⁶	5.82	5.81	6.01	5.98	0.033	ns.	ns.	ns.
pH 24 h ⁶	5.65	5.68	5.71	5.71	0.038	ns.	ns.	ns.
L* 45 min ⁷	55.0	55.0	53.8	53.7	1.83	ns.	ns.	ns.
a* 45 min ⁷	15.4	15.0	14.7	14.7	1.12	ns.	ns.	ns.
b* 45 min ⁷	5.3	5.3	5.2	5.6	0.33	ns.	ns.	ns.

Abbreviations: F2 = thickness of the backfat at 3/4 of the thoracic vertebrae from the last rib; M2 = thickness of the longissimus thoracis muscle; L* = lightness; a* = redness; b* = yellowness; ns. = not significant.

¹ basal diet without glutamine supplementation

² group supplemented with 0.5% glutamine.

³ main effect of the group (control vs supplemented).

⁴ main effect of sex (barrows vs gilts).

⁵ effect of interaction (group and sex).

⁶ pH measured after 30 min and 24 h following slaughter.

⁷ Colour parameters measured after 45 min following slaughter.

All muscles exceeded the reference CS of 100 for every EAA (Table 8). In LL, CS for Met+Cys was higher in gilts ($P = 0.015$), Val CS rose in barrows with Gln ($P = 0.003$), and interactions affected Leu and Cys CS (both $P < 0.03$). In TB, Gln increased CS for His ($P = 0.049$), Ile ($P = 0.016$), Met+Cys ($P = 0.020$) and Phe+Tyr ($P < 0.001$), while Leu CS was lower in Gln-fed pigs and control gilts versus control barrows ($P = 0.006$); His also showed a sex effect ($P = 0.047$). In SM, only Met and Met+Cys CS were sex-dependent, both higher in barrows ($P = 0.008$ and $P = 0.012$, respectively).

Cytokines and glutathione

In LL, 0.5% Gln cut IL-1 β and TNF- α levels by roughly half ($P < 0.001$; Fig. 2). Regardless of diet, TNF- α in barrows was over three times that in gilts. In TB, Gln had sex-dependent effects on IL-1 β , without changes in barrows and lowering it in gilts ($P < 0.001$), while IL-6 and TNF- α remained comparable across groups except for control gilts, which showed elevated IL-6 ($P = 0.003$) and TNF- α ($P = 0.017$). In SM, IL-6 was unchanged by either factor; IL-1 β dropped significantly in Gln-fed gilts ($P < 0.001$) but not in barrows, and TNF- α fell markedly in barrows ($P < 0.001$) with a modest decrease in gilts.

Glutathione in LL and SM was higher in barrows than gilts ($P < 0.001$; Fig. 3). Gln supplementation lowered GSH in barrows and raised it in gilts in these two muscles, whereas TB GSH levels were unaffected by either sex or diet.

Discussion

Carcass and longissimus lumborum muscle traits

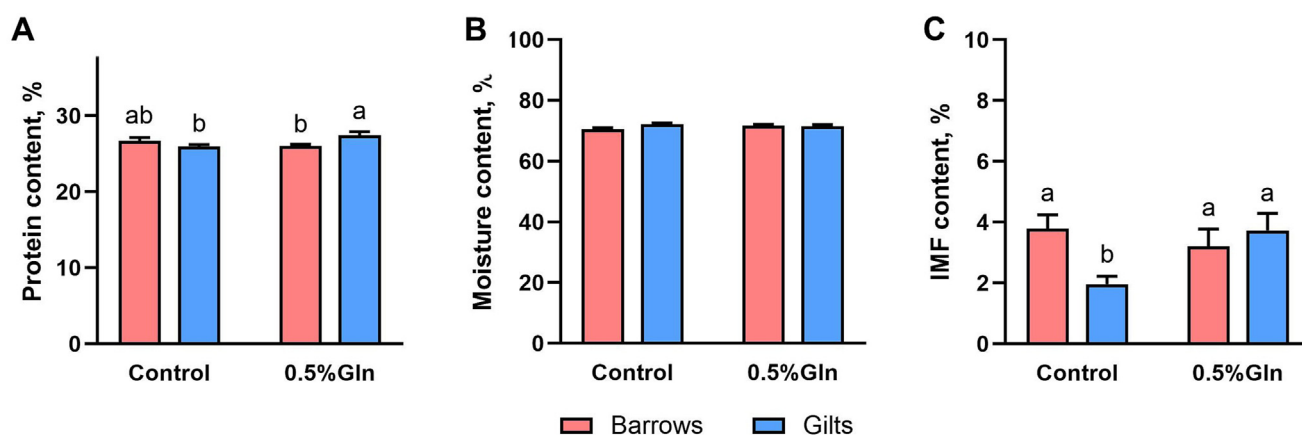
The current study evaluated the impact of 0.5% glutamine (Gln) supplementation on meat and carcass traits. In addition to muscle composition and metabolic markers, Gln supplementation also significantly influenced performance traits. As shown, pigs receiving

dietary Gln in the early postweaning period exhibited similar average daily gain, feed conversion ratio, and greater carcass weight compared to controls. Furthermore, the reduction in within-group weight variability suggests a more uniform growth pattern. These benefits likely stem from improved gut function, enhanced nutrient absorption, and reduced postweaning stress, consistent with Gln's known roles in intestinal health and immune modulation. Thus, Gln can offer practical benefits for growth performance and production efficiency.

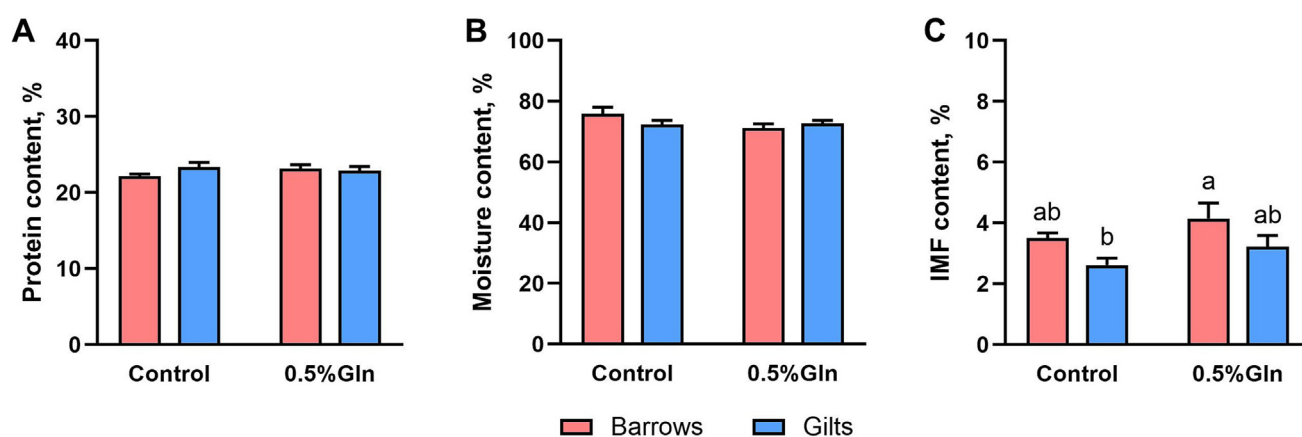
Furthermore, although the physicochemical quality (pH and colour) of LL was not significantly different across groups, there were notable changes in several carcass traits. Gln supplementation significantly increased leanness, a crucial trait for carcass quality and commercial value. The results of the conducted research not only prove that early-life dietary Gln supplementation positively influences carcass traits but also confirm the previously stated hypothesis regarding improved muscle development. The average carcass weight in this study was a bit higher than that documented in most EU countries (Dokmanovic et al., 2015). Additionally, hot carcass weight was higher in barrows than in gilts, which aligns with the general observations reported by Latorre et al. (2004) and Gispert et al. (2010), while Oliveira et al. (2015) reported no significant effect of sex on hot or cold carcass weight.

In the current study, treatment with 0.5% Gln significantly affected leanness, subcutaneous fat and muscle thickness as indicated by F2 and M2 point measurements; however, gender did not affect the aforementioned parameters. Although some previous studies have reported sex-related differences in carcass weight and subcutaneous fat (Zomeño et al., 2023), our primary focus was to assess the effect of Gln supplementation, and its interaction with sex and muscle type. Notably, these earlier studies involved Gln supplementation during the fattening or finishing phase, whereas in our study, the intervention was applied early in the postweaning period, which may underlie some of the divergent responses observed. In our study, Gln did not significantly affect pH values or meat colour in LL, regardless of sex or muscle, suggesting that glycolytic potential and postmortem lactic acid accumulation were

LL



TB



SM

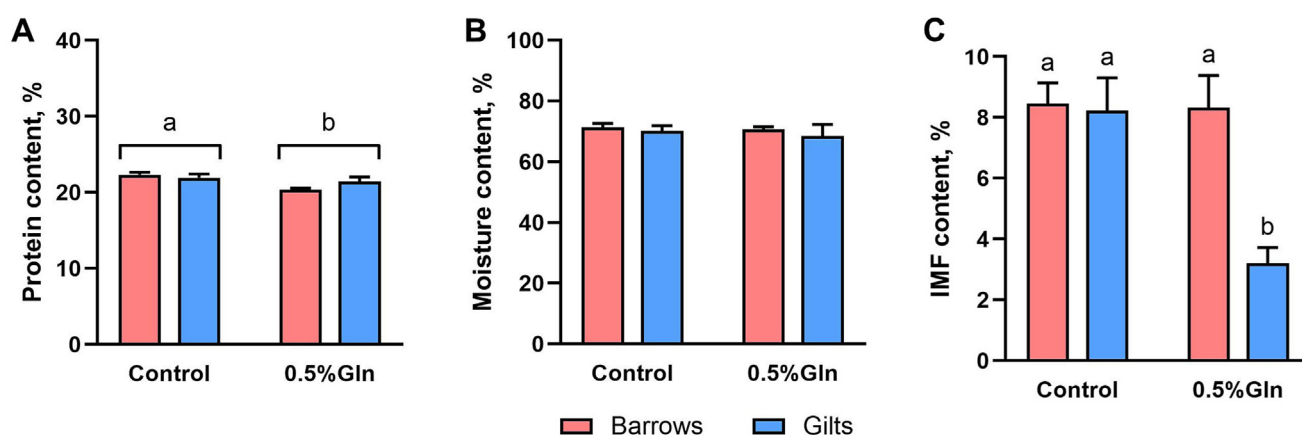


Fig. 1. The effect of glutamine (Gln) supplementation to weaner pigs (gilts and barrows) for 42 days on protein (A), moisture (B), and intramuscular fat content – IMF (C) in longissimus lumborum (LL), triceps brachii (TB) and semimembranosus (SM) muscles at the time of slaughter determined for the control and experimental group (0.5%Gln). Data are presented as least squares mean $\pm$ SEM. Indices a and b denote statistically different groups ($P < 0.05$).

not altered by the treatment. Similar conclusions were drawn by Kim et al. (2016, 2020) and Zhang et al. (2018), who found these traits to be more dependent on genotype or preslaughter conditions than on nutritional interventions. Thus, the observed meat quality traits appear to be largely unaffected by Gln supplementation.

These muscle-specific effects are likely attributable to intrinsic differences in fibre-type composition and metabolic profiles: SM exhibits a mixed oxidative-glycolytic profile, LL is predominantly

oxidative, while TB, particularly its long head, has a high proportion of glycolytic type IIB fibres, which are less responsive to Gln-driven lipid modulation (Choi and Kim, 2009).

Proximate composition

Tissue composition plays a crucial role in determining the nutritional and technological value of meat, particularly in relation to lipid content and its distribution (Duziński et al., 2015). In the pre-

Table 5The effect of Gln supplementation to weaner pigs (gilts and barrows) for 42 days on amino acid content (mg/g_{muscle}) in LL at the time of slaughter.

Variable	control ¹		0.5%Gln ²		SEM	P-value		
	Barrows	Gilts	Barrows	Gilts		Group ³	Sex ⁴	Group × Sex ⁵
Number of animals	6	6	6	6				
Thr	9.60	9.60	9.98	9.94	0.133	0.009	ns.	ns.
Val	9.60 ^a	9.80 ^a	10.56 ^b	9.84 ^a	0.131	0.001	ns.	0.002
Ile	9.07	9.18	9.44	9.35	0.089	0.006	ns.	ns.
Leu	15.90 ^a	16.20 ^a	17.47 ^b	16.24 ^a	0.277	0.010	ns.	0.014
Phe	8.16	8.32	8.61	8.50	0.119	0.015	ns.	ns.
His	8.90 ^a	9.21 ^a	10.06 ^b	9.22 ^a	0.192	0.006	ns.	0.006
Lys	18.65	18.65	19.62	19.03	0.207	0.005	ns.	ns.
Met	7.58	8.12	8.26	8.93	0.231	0.004	0.017	ns.
Cys	3.86 ^{a,b}	3.65 ^c	3.74 ^{a,c}	3.98 ^b	0.052	ns.	ns.	<0.001
Total EAA	91.3 ^a	92.7 ^{a,b}	97.7 ^c	95.0 ^{b,c}	0.700	<0.001	ns.	0.008
Asp + Asn	19.59	19.80	20.28	20.25	0.260	0.041	ns.	ns.
Ser	8.04	8.31	8.57	8.54	0.141	0.014	ns.	ns.
Glu + Gln	31.10 ^a	31.70 ^{a,b}	33.84 ^c	32.88 ^{b,c}	0.312	<0.001	ns.	0.022
Pro	7.28	7.46	7.72	7.65	0.272	ns.	ns.	ns.
Gly	8.14	8.35	8.67	8.36	0.158	ns.	ns.	ns.
Ala	11.84	12.10	12.27	12.23	0.220	ns.	ns.	ns.
Tyr	8.56	8.69	9.12	8.87	0.122	0.005	ns.	ns.
Arg	13.20	13.19	13.67	13.52	0.168	0.030	ns.	ns.
Total NEAA	107.8 ^a	109.6 ^{a,b}	114.1 ^c	112.3 ^{b,c}	0.850	<0.001	ns.	0.043
%EAA	45.87	45.83	46.13	45.84	0.179	ns.	ns.	ns.
EAA/NEAA	0.85	0.85	0.86	0.85	0.011	ns.	ns.	ns.
Total AAs	199.1 ^a	202.3 ^{a,b}	211.9 ^c	207.3 ^{b,c}	1.380	<0.001	ns.	0.010

Abbreviations: AAs = amino acids; LL = longissimus lumborum; Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid (aspartate); Cys = cysteine; Gln = glutamine; Glu = glutamic acid (glutamate); Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Tyr = tyrosine; Val = valine; EAA = essential amino acids; NEAA = nonessential amino acids; ns. = not significant.

Values within a row with different superscripts differ significantly at $P < 0.05$.

¹ basal diet without glutamine supplementation.

² group supplemented with 0.5% glutamine.

³ main effect of the group (control vs supplemented).

⁴ main effect of sex (barrows vs gilts).

⁵ effect of interaction (group and sex).

Table 6The effect of Gln supplementation to weaner pigs (gilts and barrows) for 42 days on amino acid content (mg/g_{muscle}) in TB at the time of slaughter.

Variable	control ¹		0.5%Gln ²		SEM	P-value		
	Barrows	Gilts	Barrows	Gilts		Group ³	Sex ⁴	Group × Sex ⁵
Number of animals	6	6	6	6				
Thr	10.34	9.90	9.88	9.77	0.171	ns.	ns.	ns.
Val	10.05	10.25	9.92	9.89	0.178	ns.	ns.	ns.
Ile	8.97	8.92	8.97	9.08	0.147	ns.	ns.	ns.
Leu	18.44	17.29	16.67	16.66	0.306	<0.001	ns.	ns.
Phe	8.90	8.50	8.46	8.41	0.126	ns.	ns.	ns.
His	6.92	7.90	7.75	8.06	0.320	ns.	ns.	ns.
Lys	20.05	19.25	18.87	18.91	0.260	0.008	ns.	ns.
Met	7.88	8.22	8.89	9.01	0.517	ns.	ns.	ns.
Cys	3.87	3.68	3.93	3.95	0.282	ns.	ns.	ns.
Total EAA	95.4	93.9	93.3	93.7	1.151	ns.	ns.	ns.
Asp + Asn	20.40	19.60	19.93	19.58	0.300	ns.	ns.	ns.
Ser	8.82	8.49	8.57	8.38	0.134	ns.	ns.	ns.
Glu + Gln	37.75 ^a	35.10 ^b	35.23 ^b	34.94 ^b	0.444	0.007	0.004	0.016
Pro	8.87	8.38	8.23	8.05	0.363	ns.	ns.	ns.
Gly	8.85	9.68	9.17	8.42	0.352	ns.	ns.	ns.
Ala	12.75	12.64	12.46	12.09	0.208	ns.	ns.	ns.
Tyr	8.27	8.18	8.24	8.35	0.120	ns.	ns.	ns.
Arg	14.25 ^a	16.64 ^b	13.57 ^a	13.44 ^a	0.260	<0.001	<0.001	<0.001
Total NEAA	119.9	118.7	115.4	113.2	1.401	0.002	ns.	ns.
%EAA	44.31	44.17	44.71	45.29	0.261	0.008	ns.	ns.
EAA/NEAA	0.80	0.79	0.81	0.83	0.089	0.008	ns.	ns.
Total AAs	215.4	212.6	208.7	207.0	2.303	0.015	ns.	ns.

Abbreviations: AAs = amino acids; TB = triceps brachii; Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid (aspartate); Cys = cysteine; Gln = glutamine; Glu = glutamic acid (glutamate); Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Tyr = tyrosine; Val = valine; EAA = essential amino acids; NEAA = nonessential amino acids; ns. = not significant.

Values within a row with different superscripts differ significantly at $P < 0.05$.

¹ basal diet without glutamine supplementation.

² group supplemented with 0.5% glutamine.

³ main effect of the group (control vs supplemented).

⁴ main effect of sex (barrows vs gilts).

⁵ effect of interaction (group and sex).

Table 7The effect of Gln supplementation to weaner pigs (gilts and barrows) for 42 days on amino acid content (mg/g_{muscle}) in SM at the time of slaughter.

Variable	control ¹		0.5%Gln ²		SEM	P-value		
	Barrows	Gilts	Barrows	Gilts		Group ³	Sex ⁴	Group × Sex ⁵
Number of animals	6	6	6	6				
Thr	9.70a,b	10.05a,b	9.20 ^a	10.51b	0.209	ns.	0.001	0.037
Val	10.08	10.50	9.52	10.90	0.321	ns.	0.010	ns.
Ile	8.72	9.37	8.34	9.41	0.182	ns.	<0.001	ns.
Leu	16.45	17.35	15.82	17.97	0.351	ns.	<0.001	ns.
Phe	8.14	8.59	7.86	8.85	0.170	ns.	<0.001	ns.
His	8.71	8.31	7.75	8.51	0.369	ns.	ns.	ns.
Lys	15.04	19.64	17.83	20.10	0.891	ns.	0.001	ns.
Met	8.51	8.45	8.61	8.75	0.248	ns.	ns.	ns.
Cys	3.59	3.89	3.88	3.83	0.200	ns.	ns.	ns.
Total EAA	88.9	96.1	88.8	98.8	1.387	ns.	<0.001	ns.
Asp + Asn	19.20	20.09	18.24	20.30	0.369	ns.	<0.001	ns.
Ser	8.27	8.58	7.92	8.88	0.182	ns.	0.003	ns.
Glu + Gln	33.05	35.90	32.16	36.13	0.800	ns.	<0.001	ns.
Pro	7.30	8.28	7.40	8.18	0.290	ns.	0.006	ns.
Gly	8.80	9.44	8.58	9.26	0.202	ns.	0.003	ns.
Ala	11.83	12.55	11.46	12.80	0.254	ns.	0.006	ns.
Tyr	8.33	8.56	7.76	8.79	0.181	ns.	0.003	ns.
Arg	12.65	13.55	12.34	13.87	0.233	ns.	<0.001	ns.
Total NEAA	109.4	116.9	105.9	118.2	1.700	ns.	<0.001	ns.
%EAA	44.83	45.11	45.61	45.55	0.228	0.015	ns.	ns.
EAA/NEAA	0.81	0.82	0.84	0.84	0.011	0.015	ns.	ns.
Total AAs	198.3	213.1	194.7	217.0	2.986	ns.	<0.001	ns.

Abbreviations: AAs = amino acids; SM = semimembranosus; Ala = alanine; Arg = arginine; Asn = asparagine; Asp = aspartic acid (aspartate); Cys = cysteine; Gln = glutamine; Glu = glutamic acid (glutamate); Gly = glycine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Pro = proline; Ser = serine; Thr = threonine; Tyr = tyrosine; Val = valine; EAA = essential amino acids; NEAA = nonessential amino acids; ns. = not significant.

Values within a row with different superscripts differ significantly at $P < 0.05$.

¹ basal diet without glutamine supplementation.

² group supplemented with 0.5% glutamine.

³ main effect of the group (control vs supplemented).

⁴ main effect of sex (barrows vs gilts).

⁵ effect of interaction (group and sex).

sent study, Gln supplementation led to muscle-specific and sex-dependent alterations in protein, IMF, and moisture content. For example, in LL, Gln supplementation was associated with increased protein content in gilts, while changes in IMF were more pronounced in other muscle types and varied by sex. These findings align with previous reports on Polish crossbred pigs (PLW × PL) indicating sex-related differences in tissue composition (Jacyno et al., 2002; Jankowiak et al., 2018, 2021). Although values observed in TB and SM varied from those in earlier studies (Prusa et al., 1989; Wójciak et al., 2021), these differences were not the primary focus of our study. Rather, we aimed to explore how nutrient composition within each muscle was modulated by Gln supplementation and its interaction with sex. Notably, previous research has reported that dietary glutamate or Gln can influence lipid deposition and protein metabolism (Zhou et al., 2014; Hu et al., 2017). Our findings suggest that these effects are not uniform across muscle types, highlighting the importance of evaluating muscle-specific responses rather than direct inter-muscle comparisons. This is consistent with evidence that muscle metabolism is influenced by differential myogenic activity and adipogenesis during development (Liu et al., 2009; Herault et al., 2014).

In our study, sex- and muscle-specific differences in IMF and protein content following Gln supplementation point to underlying variation in local metabolic capacity and nutrient sensitivity (Liu et al., 2009; Herault et al., 2014). For instance, LL of gilts showed increased protein content, which may reflect a greater anabolic responsiveness or reduced tendency for lipid accumulation in this specific muscle-sex combination. This aligns with previous findings showing that glutamic acid can reduce backfat thickness and improve fatty acid composition (Hu et al., 2017), while monosodium glutamate has been reported to increase IMF content (Zhou

et al., 2014). However, our results did not reveal a consistent effect of Gln on overall carcass fatness or IMF content across muscles. This discrepancy with earlier studies may stem from differences in the chemical form of glutamate used (glutamine vs. free glutamic acid or its salts), the physiological status of the animals, the duration of supplementation, or the timing of sample collection (e.g., postweaning vs. slaughter). Notably, our study revealed that the effects of Gln were significantly modulated by both sex and muscle type, suggesting that the metabolic responsiveness to Gln is more context-dependent than previously assumed. These findings point towards a role for local regulatory factors, such as sex hormones, muscle fibre composition, and region-specific expression of nutrient transporters or metabolic enzymes, in shaping the tissue-specific outcomes of Gln supplementation. Potential molecular pathways involved may include mTOR (which regulates protein synthesis) and SREBP (involved in lipid metabolism), both of which are known to be influenced by amino acid availability. Taken together, our results support the concept of developmental programming, whereby early-life nutritional interventions can induce long-term, tissue- and sex-specific metabolic effects.

Amino acids

Amino acids play a fundamental role in muscle growth and meat quality, acting as building blocks for protein synthesis and contributors to flavour and nutritional value (Wu, 2009; Guo et al., 2020; Ma et al., 2020). In our study, the concentrations of Glu, Leu, and Lys were within the range previously reported in commercial and local pig breeds (Zhang et al., 2019; Siemiński et al., 2023). This suggests that dietary Gln supplementation did not dramatically alter the overall amino acid profile of muscle tis-

Table 8

The effect of Gln supplementation to weaner pigs (gilts and barrows) for 42 days on the CS (%) index of LL, TB, and SM at the time of slaughter.

CS	control ¹		0.5%Gln ²		SEM	P-value		
	Barrows	Gilts	Barrows	Gilts		Group ³	Sex ⁴	Group × Sex ⁵
Number of animals	6	6	6	6				
LL								
His	298.2	303.6	316.7	296.6	6.42	ns.	ns.	ns.
Ile	151.8	151.2	148.5	150.2	1.42	ns.	ns.	ns.
Leu	135.2 ^a	135.7 ^a	139.7 ^a	132.7 ^b	1.91	ns.	ns.	0.029
Lys	208.2	204.8	205.7	203.9	2.11	ns.	ns.	ns.
Met	237.9	250.7	243.7	269.3	7.00	ns.	0.013	ns.
Cys	323.2 ^a	300.7 ^{a,b}	294.1 ^b	320.2 ^a	4.58	ns.	ns.	0.006
Met+Cys	261.2	264.3	257.4	283.2	5.43	ns.	0.015	ns.
Phe+Tyr	220.9	221.2	220.1	220.4	1.09	ns.	ns.	ns.
Thr	209.6	206.3	204.7	208.5	2.13	ns.	ns.	ns.
Val	123.5 ^a	124.2 ^{a,b}	127.7 ^b	121.6 ^a	1.03	ns.	0.013	0.003
TB								
His	214.0	247.7	247.6	260.6	11.00	0.049	0.047	ns.
Ile	138.8	139.82	143.3	146.3	2.11	0.016	ns.	ns.
Leu	145.1 ^a	137.9 ^{a,b}	135.3 ^b	136.3 ^b	1.64	0.011	ns.	0.006
Lys	206.8	201.2	200.9	203.0	1.23	ns.	ns.	ns.
Met	228.6	241.7	265.9	271.8	15.14	0.020	ns.	ns.
Cys	299.5	288.5	313.8	316.8	21.00	ns.	ns.	ns.
Met+Cys	247.9	254.5	279.0	284.0	11.89	0.020	ns.	ns.
Phe+Tyr	209.7	206.4	210.6	213.1	1.57	0.030	ns.	ns.
Thr	208.8	202.4	205.7	205.3	2.60	ns.	ns.	ns.
Val	119.6	123.6	121.8	122.6	1.88	ns.	ns.	ns.
SM								
His	292.8	259.8	265.3	261.8	11.51	ns.	ns.	ns.
Ile	146.6	146.6	142.8	144.5	2.41	ns.	ns.	ns.
Leu	140.8	137.9	137.7	140.3	2.72	ns.	ns.	ns.
Lys	167.7	204.9	203.6	205.8	8.51	ns.	ns.	ns.
Met	268.2	247.8	276.3	252.3	7.47	ns.	0.008	ns.
Cys	301.9	304.1	331.5	295.4	16.71	ns.	ns.	ns.
Met+Cys	277.4	263.2	291.4	264.1	7.50	ns.	0.012	ns.
Phe+Tyr	218.5	211.8	211.2	213.9	2.88	ns.	ns.	ns.
Thr	212.9	205.0	205.4	210.4	3.99	ns.	ns.	ns.
Val	130.4	126.2	125.3	128.7	3.41	ns.	ns.	ns.

Abbreviations: SM = semimembranosus; TB = triceps brachii; LL = longissimus lumborum; CS = chemical score; Cys = cysteine; Gln = glutamine; His = histidine; Ile = isoleucine; Leu = leucine; Lys = lysine; Met = methionine; Phe = phenylalanine; Thr = threonine; Tyr = tyrosine; Val = valine; ns. = not significant.

Values within a row with different superscripts differ significantly at $P < 0.05$.

¹ basal diet without glutamine supplementation.

² group supplemented with 0.5% glutamine.

³ main effect of the group (control vs supplemented).

⁴ main effect of sex (barrows vs gilts).

⁵ effect of interaction (group and sex).

sue, reinforcing the notion that AA composition may be primarily governed by genetic background and muscle-specific metabolism rather than by short-term nutritional interventions.

Nevertheless, we observed muscle- and sex-specific differences in AAs content following Gln supplementation. In LL, barrows showed a notable increase in total AAs and NEAA content, while in TB, changes were observed in EAA/NEAA ratios and %EAA. In SM, Gln supplementation primarily affected %EAA and EAA/NEAA balance in gilts. These findings indicate that the response to Gln is modulated by both anatomical and physiological context.

Several previous studies have reported minimal or inconsistent effects of sex on the AA profile of the longissimus thoracis et lumborum (Okrouhlá et al., 2006; Nevrlka et al., 2023; Ludwiczak et al., 2024), although some identified subtle sex-based differences in specific AAs (e.g., (Cai et al., 2010)). Our results extend this knowledge by showing that sex does influence AA composition when interacting with muscle type and dietary Gln. Notably, the strongest effects were seen in barrows, suggesting that hormonal status and postnatal muscle development may modulate the sensitivity to Gln.

These findings are in line with previous reports of increased Arg and Glu following Gln supplementation in growing pigs (Wang et al., 2024) and increased Arg, His, and Tau after glutamate and

alanine administration (Hu et al., 2017). Additionally, Glu remained the most abundant AAs across all muscles and sexes in our study, which is expected given the porcine liver's role in synthesising Glu from Gln and branched-chain amino acids via low BCAT activity (Zhang et al., 2021).

While the overall AA profile remained within normal ranges, slight but significant variations across muscles and sexes point to underlying differences in metabolic activity, fibre composition, and protein turnover. These observations support the complexity of skeletal muscle metabolism and reinforce the importance of considering both anatomical site and sex when designing early-life nutritional strategies. Importantly, our study adds novel insight by including two under-represented muscles (TB and SM), for which limited AA data have been published to date. The results suggest that postweaning Gln supplementation can differentially influence AAs profiles in a muscle- and sex-specific manner, consistent with tissue-specific metabolic regulation.

Cytokines and glutathione

Glutamine is known to support gut health and immune function, particularly during the stressful weaning period. A large proportion of dietary Gln is converted to α -ketoglutarate, providing

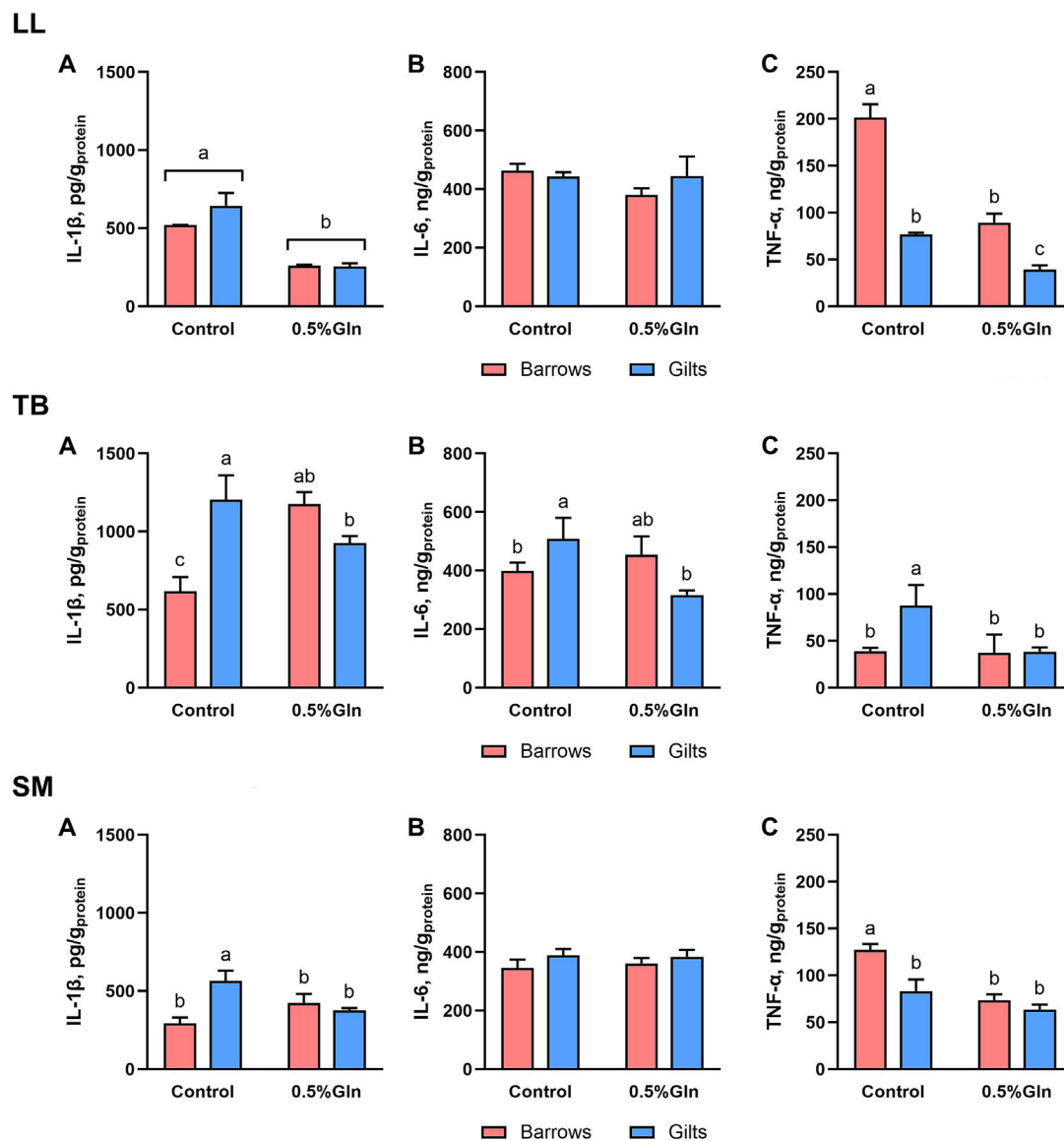


Fig. 2. The effect of glutamine (Gln) supplementation to weaner pigs (gilts and barrows) for 42 days on cytokines: (A) interleukin-1 beta (IL-1 β), (B) interleukin-6 (IL-6), and (C) tumour necrosis factor alpha (TNF- α) in longissimus lumborum (LL), triceps brachii (TB) and semimembranosus (SM) muscles at the time of slaughter determined for the control and experimental group (0.5% Gln). Data are presented as least squares mean $\pm$ SEM. Indices a, b, and c denote statistically different groups ($P < 0.05$).

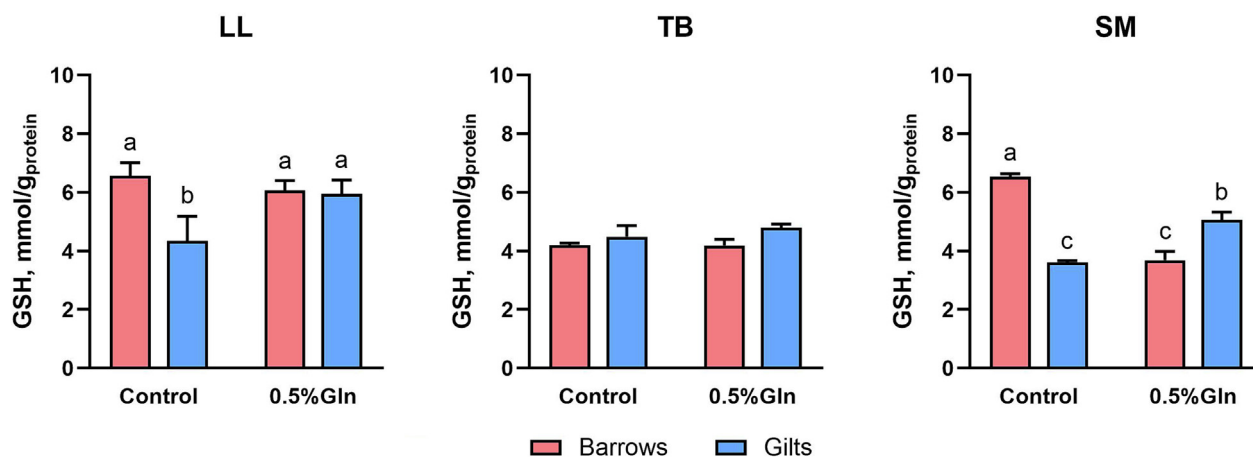


Fig. 3. The effect of glutamine (Gln) supplementation to weaner pigs (gilts and barrows) for 42 days on glutathione (GSH) in longissimus lumborum (LL), triceps brachii (TB) and semimembranosus (SM) muscles at the time of slaughter determined for the control and experimental group (0.5% Gln). Data are presented as least squares mean $\pm$ SEM. Indices a, b, and c denote statistically different groups ($P < 0.05$).

energy to enterocytes, while also promoting the synthesis of GSH, a critical antioxidant (Wang et al., 2008; Watford, 2015). Weaning stress induces oxidative and inflammatory responses, which can impair growth and muscle development (Kim and Duarte, 2021; Zheng et al., 2021). In our study, early-life Gln supplementation resulted in muscle- and sex-specific changes in GSH levels. In gilts, GSH increased in LL and SM, suggesting enhanced antioxidant capacity. In contrast, in barrows, GSH levels were higher overall, and Gln caused a decrease in LL and SM, indicating possible overcompensation or differential metabolic use of Gln. These findings confirm the hypothesis that Gln modulates antioxidant defence in a sex-dependent manner (Hanczakowska and Niwińska, 2013; Sychoy et al., 2025). GSH plays a central role in maintaining muscle integrity under stress, acting not only as a potent antioxidant but also participating in repair and regeneration processes (Baldelli et al., 2019; Averill-Bates, 2023).

The pro-inflammatory cytokine IL-1 β decreased in both LL and SM following Gln supplementation, with a notable reduction in gilts' TB as well. IL-1 β is a key mediator of inflammation and muscle catabolism, and its suppression aligns with observed improvements in muscle antioxidant status (Londhe and Guttridge, 2015; Luo et al., 2024). Similarly, IL-6 levels declined in TB of gilts after Gln, which may relate to improved IMF deposition, considering IL-6's known role in lipid metabolism and lipolysis (Kim et al., 2004; Pothakam et al., 2021). Although the exact pathways remain unclear, this suggests a regulatory link between immune signalling and lipid accumulation in muscle, modulated by Gln.

TNF- α , another pro-inflammatory cytokine involved in lipid metabolism and insulin resistance, was also reduced following Gln supplementation. Collectively, these cytokine and GSH changes support the proposed anti-inflammatory and antioxidant role of Gln in muscle tissue, particularly under postweaning stress conditions. Importantly, these effects were not uniform across sexes or muscles, emphasising the complexity of Gln's biological actions.

In conclusion, the results of this study confirm our initial hypothesis that early-life dietary Gln supplementation can enhance growth performance, positively affect the amino acid and proximate composition of pork, and modulate IMF deposition in a muscle- and sex-specific manner. By improving carcass characteristics, increasing protein content, optimising the AAs profile, and exerting immunomodulatory and antioxidant effects (as evidenced by reduced pro-inflammatory cytokine levels and altered GSH concentrations), supplementation with Gln during the weaner period has been shown to support both health and meat quality in pigs at slaughter.

Importantly, these effects were both muscle- and sex-specific.

These findings highlight the metabolic complexity of Gln's action and suggest that its efficacy depends on tissue type and physiological context. While our study focused on the effects of early Gln supplementation on meat quality, we acknowledge that demonstrating its economic viability requires further applied research under commercial conditions to comprehensively assess the cost-effectiveness of Gln supplementation.

Ethics approval

The study received approval from the Local Ethics Committee on Animal Experimentation of the University of Life Sciences in Lublin, Poland (51/2017).

Data and model availability statement

The data/models were not deposited in an official repository but are available from the authors upon request.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT 4.0 and Grammarly in order to make grammar and language corrections. After using these services, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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Declaration of interest

None.

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