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Original Research Article

Replacing rice straw with peanut vine and *Broussonetia papyrifera* silage in beef cattle feed reduced the use of soybean meal

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The present study investigated whether replacing dietary rice straw with peanut vine (PEV) and *Broussonetia papyrifera* silage (BPS) reduces the use of soybean meal and explored its effects on the growth performance, blood biochemical indicators, serum metabolomics, and meat quality of fattening bulls. Forty-five Simmental crossbred bulls (initial body weight = 484.29 ± 8.49 kg) were randomly allotted into three dietary treatment groups ($n = 15$): (1) CON, 5% rice straw (DM basis); (2) PEV, 5% peanut vine (DM basis); and (3) BPS, 5% *B. papyrifera* silage (DM basis). The remaining roughage for all three treatment groups was supplemented with 25% corn silage (DM basis). The experiment lasted for 123 d, with the first 14 d serving as an adaptive period. Throughout the experiment, dietary BPS decreased the average daily dry matter intake ($P < 0.001$) and feed cost ($P < 0.001$). Serum metabolomics analysis showed that PEV affected the phenylalanine, tyrosine, and tryptophan biosynthesis pathways ($P = 0.021$) and lysine degradation pathway ($P = 0.042$), whereas BPS affected the phenylalanine, tyrosine and tryptophan biosynthesis pathways ($P = 0.004$), lysine degradation pathway ($P = 0.012$), and serotonergic synapse pathway ($P < 0.001$). Regarding meat quality, the redness ($P = 0.025$) and hue angle values ($P < 0.001$) of the longissimus dorsi muscle were lower in the BPS group than in the CON and PEV groups. The yellowness of the longissimus dorsi muscle was lower in the BPS group than in the PEV group ($P = 0.024$), and the shear force was lower in the PEV group than in the BPS group ($P = 0.014$). However, lysine content in beef was higher in the BPS group than in the CON group ($P = 0.005$). In conclusion, replacing rice straw with PEV and BPS reduced the use of soybean meal but had no adverse effects on growth performance. BPS affected the amino acid metabolism of bulls, thus decreasing feed intake and increasing the lysine content in meat. The PEV group showed better meat quality than the BPS group.

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1. Introduction

In recent years, with improved living standards and an in-depth understanding of nutrition, the demand for beef has increased rapidly (Zhao et al., 2022). However, the development of the beef cattle industry in China is limited by the shortage of roughage and protein feed resources (Song et al., 2017; Wang et al., 2022). The use of alternative feed resources may be the key to reducing costs and diversifying the ruminant diet (El et al., 2021; He et al., 2022).

Peanut (*Arachis hypogaea* L.), an oil crop with significant economic value, is widely cultivated worldwide (Cui et al., 2022). China, the world's largest peanut producer, produced 18.20 million tons of peanuts in 2022 (Liu et al., 2020). Peanut vine (PEV) is the main byproduct of the peanut industry, accounting for 60% to 65% of the biomass in peanut production (Zhao et al., 2012). Previously, PEV was considered a potential pollutant in peanut production (Zhang et al., 2016). The exploration of alternative feed resources in recent years has revealed that, owing to its high crude protein (CP) concentration (12%), PEV has a high feed value for ruminants (Zhang et al., 2022). Wang et al. (2016) reported that incorporating

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PEV instead of corn silage in the feed of lactating cows did not decrease the production performance and dairy product quality but decreased feed costs. Similarly, the economic benefit of using two roughage sources (PEV and alfalfa meal) in the feed of Boer × Macheng crossbred goats was reported to be higher than using alfalfa meal alone (Suo et al., 2021).

Paper mulberry (*Broussonetia papyrifera* L.) belongs to the Moraceae family and is a perennial woody tree (Tang et al., 2022). Owing to its fiber length, various bioactive substances, and heavy metal resistance, paper mulberry has been widely used in paper making, medicine, and ecological afforestation (Peng et al., 2019). Moreover, owing to its high yield potential and CP content, it has garnered significant interest as a potential feed resource (Meier et al., 2013; Wu et al., 2022). However, the protein structure of paper mulberry is complex; the digestion, absorption and utilization rates of nutrients are low; and fresh leaves are hard to preserve. Using ensiling technology softens lignin and fiber substances, extending their storage life. Hao et al. (2020) studied the effect of BPS on the production performance of high-producing lactating dairy cows and observed that 4.5% BPS in the feed increased fat-corrected milk yield by 3.5%, but milk yield decreased when 13.5% BPS was used. He et al. (2021) reported that using *Broussonetia papyrifera* silage (BPS) instead of alfalfa hay in the basic diet of goats did not affect the growth performance. Similarly, another study showed that the replacement of corn silage and wheat straw with BPS did not affect the growth performance of beef cattle (Hu et al., 2022).

At present, the feed production industry is facing challenges such as a shortage of protein feed and high costs of soybean meal. PEV and BPS have high protein content, and their amino acid composition is different from that of soybean meal. We hypothesized that the reasonable addition of PEV and BPS to substitute rice straw in the feed of Simmental crossbred bulls may not only reduce the use of soybean meal but also improve beef quality through effects on the amino acid metabolism. To verify this hypothesis, we subjected fattening Simmental crossbred bulls to three dietary treatments and evaluated their effects on the growth performance, blood biochemical indicators, serum metabolomics, and meat quality of the bulls.

2. Materials and methods

2.1. Animal ethics

All the experimental procedures applied in this study were reviewed and approved by the Animal Care and Use Committee of China Agricultural University (Permit No. DK3178).

2.2. Experimental animals, design, and management

Forty-five healthy Simmental crossbred bulls with similar body weight (BW; initial BW = 484.29 ± 8.49 kg) were selected and randomly divided into three treatment groups (15 animals per group) according to a single-factor completely randomized design. The three dietary treatments were as follows: (1) CON group, 5% rice straw (DM basis; initial BW = 483.85 ± 13.66 kg); (2) PEV group, 5% PEV (DM basis; initial BW = 478.64 ± 17.20 kg); (3) BPS group, 5% *B. papyrifera* wrapped silage (DM basis; initial BW = 490.45 ± 14.55 kg). PEV and BPS were purchased from Heze, Shandong, China. The processing method used to obtain BPS included crushing *B. papyrifera* and then wrapping it for silage. The standard nutrient composition of these three roughage sources is shown in Table 1. The total mixed ration (TMR) formula was based on the nutritional composition of each feedstuff, following the principle of iso-energy and iso-nitrogen and according to the recommendations of NASEM (2016) (Table 2). The objective of the TMR formulation was to meet the average daily gain (ADG) target of 0.80 kg/d for 500 kg Simmental bulls.

This study was conducted at Longshengyuan Livestock Farm (Linyi, Shandong, China; 118.71 °E, 35.62 °N) from February to June

Table 1
Analysis of the standard nutrient composition of rice straw, PEV, and BPS (%, DM basis).

Item	Rice straw	PEV	BPS
Dry matter	93.89	93.26	18.97
Crude protein	6.07	7.35	14.71
Ether extract	0.34	0.67	3.40
Neutral detergent fiber	72.05	58.73	49.40
Acid detergent fiber	45.90	52.44	37.41
Ash	10.44	7.80	12.92

PEV = peanut vine; BPS = *Broussonetia papyrifera* wrapped silage.

Table 2
Ingredients and chemical composition of the TMR (%, DM basis).

Item	CON	PEV	BPS
Ingredients			
Corn grain	50.50	53.00	51.50
Soybean meal	8.50	6.50	6.00
Brewer's grain	6.00	5.50	7.50
Salt	1.00	1.00	1.00
Calcium hydrophosphate	1.00	1.00	1.00
Limestone	1.00	1.00	1.00
$NaHCO_3$	1.00	1.00	1.00
Vitamin premix[1]	1.00	1.00	1.00
Whole plant corn silage	25.00	25.00	25.00
Rice straw	5.00		
PEV		5.00	
BPS			5.00
Total	100.00	100.00	100.00
Nutrient level			
DM	66.25	66.48	61.53
CP	12.03	11.95	12.02
NDF	21.50	20.33	20.89
ADF	11.24	11.30	10.96
Ash	3.80	3.57	3.85
EE	3.71	3.75	4.00
Aspartic acid	0.88	0.84	0.93
Threonine	0.41	0.40	0.44
Serine	0.49	0.49	0.53
Glutamic acid	2.02	1.97	2.14
Proline	0.86	0.87	0.90
Glycine	0.45	0.44	0.48
Alanine	0.73	0.71	0.75
Cystine	0.19	0.19	0.20
Valine	0.57	0.54	0.59
Methionine	0.20	0.20	0.20
Isoleucine	0.41	0.41	0.45
Leucine	1.08	1.08	1.15
Tyrosine	0.27	0.28	0.33
Phenylalanine	0.54	0.52	0.58
Histidine	0.27	0.27	0.28
Lysine	0.46	0.45	0.49
Arginine	0.52	0.49	0.56
NEm,[2] MJ/kg	7.40	7.44	7.44
NEg,[2] MJ/kg	5.06	5.18	5.14

CON = control group; PEV = peanut vine group; BPS = *Broussonetia papyrifera* wrapped silage group; DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; EE = ether extract; NEm = net energy required for maintenance; NEg = net energy required for gain.

[1] Premix (per kg of DM) contains 150,000 to 450,000 IU vitamin A acetate; 40,000 to 120,000 IU vitamin D_3; 400 mg DL-α-tocopherol acetate; 250 to 750 mg copper; 1000 to 5000 mg of iron; 1000 to 3000 mg manganese; 1500 to 3700 mg of zinc; 10% to 25% calcium; 0.3% total phosphorus; 15% to 30% sodium chloride.

[2] NEm and NEg were calculated according to the guidelines of NASEM (2016), whereas the others (DM, CP, NDF, ADF, Ash, EE, and amino acids) were measured values.

2019. All experimental animals were raised individually with a tie to the feed bunk (each bunk provided ample space for bulls to move around without the need for climbing, fighting, or competing for feed). The beef bunks were cleaned daily to ensure a clean and hygienic environment. The experiment lasted for 123 d with the first 14 d for adaptation, during which ear labeling, insect repellent application, weighing, and other treatments were performed, and the formal trial period was 109 d. Throughout the experiment, all bulls were fed twice daily at 06:00 and 14:00, with ad libitum access to feed, ensuring a minimum of 5% residual feed per day. Additionally, water was provided once a day at 10:00.

2.3. Sample collection and analysis

2.3.1. Growth performance and economic benefits

The feed intake was recorded monthly, specifically, the feeding and refusal amount of each bull during the test period from d 29 to d 30, d 59 to d 60, and d 89 to d 90 were recorded. In addition, the feeding and refusal amount of each bull two days before weighing (d 58 to d 59, and d 107 to d 108) were also measured. Samples of individual ingredients, both TMR and refusals were collected biweekly and frozen for subsequent analysis. Feed samples were analyzed for determining dry matter (DM; method 934.01; AOAC, 2005), CP (Method 968.06; AOAC, 2005), ether extract (EE; method 920.39; AOAC, 2005), and ash (Method 942.05; AOAC, 2005). The contents of neutral detergent fibre (NDF) and acid detergent fibre (ADF) were performed using a filter bag and fiber analyzer (Ankom A2000i, Ankom, New York, USA) (Van Soest et al., 1991). Amino acid levels were quantified using an amino acid analyzer (Hitachi L8900; Hitachi, Tokyo, Japan) according to the sodium metabisulfite method (Method 994.12; AOAC, 2005).

On d 0, d 60, and d 109, all bulls were fasted for 12 h before the measurement of BW. The ADG (kg /d), dry matter intake (DMI; kg/d), and feed efficiency were calculated according to the following formulas:

$$\mathrm{ADG} = (\mathrm{BW_n} - \mathrm{BW_m}) / (\mathrm{n} - \mathrm{m}),$$

where n and m represent the n-th day and the m-th day of the experiment, respectively.

$$\text{Feed efficiency} = \mathrm{ADG}/\mathrm{DMI}$$

Economic benefit indicators, such as feed cost, weight gain income, and profit return, were determined using separate formulas:

$$\text{Feed cost (¥)} = \text{DMI (kg / d)} \times \text{Unit price}_{\text{TMR}} \text{ (¥ / kg)} \times \text{Trial period (d)},$$

$$\text{Weight gain income (¥)} = \text{ADG (kg / d)} \times \text{ Trial period (d)} \times \text{Unit price}_{\text{live cattle}} \text{ (¥ / kg)},$$

$$\text{Profit return (¥)} = \text{Weight gain income (¥)} - \text{ Feed cost (¥)}.$$

The unit prices for TMR (%, DM basis) of the three treatment groups were as follows: CON (¥2.02/kg), PEV (¥1.98/kg), and BPS (¥1.98/kg). The unit price for live cattle was ¥31.00/kg.

2.3.2. Blood sample collection and analysis

Blood samples were collected on d 109 of the experiment from the caudal vein of 45 bulls (n = 15) before morning feeding. The blood samples were stored at room temperature for approximately 30 min and then centrifuged at 3000 × g at 4 °C for 15 min to obtain serum, which was stored at − 80 °C for further analysis. Blood metabolites, including glucose (GLU) were quantified using a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China) and an ultraviolet visible spectrophotometer (UV-1100, Shanghai Tianmei Scientific Instrument Co., Ltd, Shanghai, China). Cholesterol (CHO), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were quantified using a commercial kit (Jiancheng Bioengineering Institute, Nanjing, China) and a clinical autoanalyzer (Hitachi 7600; Hitachi, Tokyo, Japan). Amino acid levels were quantified using an amino acid analyzer (Hitachi L8900; Hitachi, Tokyo, Japan).

2.3.3. Nontargeted metabolomic analysis of serum samples

Ten biological replicates from each group were used for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, which was performed by Majorbio (Majorbio Biotech Co., Ltd., Shanghai, China) for nontargeted metabolomic analysis. In brief, serum metabolites were extracted by treating the serum samples with 400 μL of methanol: acetonitrile (1:1, v/v) containing an internal standard solution and ultrasonicating them at 40 kHz for 30 min at 5 °C. After being placed at −20 °C for 30 min, the samples were centrifuged at 13,000 × g at 4 °C for 15 min and then concentrated under N_2 gas. In addition, prior to sample testing, blank samples were analyzed to ensure system stability and to exclude operational deviations. Quality control (QC) samples were prepared by pooling the supernatant from all samples (20 μL per sample) to monitor the stability of the analyses.

Chromatographic separation of metabolites was performed using an ExionLC AD System system (Sciex, Framingham, Massachusetts, USA) equipped with a RP column (ACQUITY BEH C18 column 2.1 × 100 mm, 1.7 μm; Waters Corporation, Milford, Massachusetts, USA). The separation process was carried out over 16 min, utilizing a gradient method for the separation. The mobile phases were solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile: isopropanol 1:1, v/v). The sample injection volume was 20 μL, the flow rate was 0.4 mL/min, and the column temperature was 40 °C. Samples were stored at 4 °C throughout the analysis period. The detailed procedure of the chromatographic separation is shown in Table S1. The mass spectrometric data were collected using an ABSCIEX-Triple TOF 5600 Mass Spectrometer (Sciex, Framingham, Massachusetts, USA) equipped with an electrospray ionization source operating in either positive or negative ion mode. Mass spectrometry signals were acquired using both positive and negative ion scan modes. Specifically, two separate runs were conducted: one in positive mode and another in negative mode, with ion spray voltage settings detailed in Table S2. Collision-Induced Dissociation (CID) was the type of fragmentation employed, and both MS and MS/MS secondary fragmentation data were acquired to ensure a comprehensive analysis of the metabolites present.

The raw data were uploaded into Progenesis QI 2.3 (Nonlinear Dynamics, Waters, USA) for peak detection and alignment, with the mass tolerance set at 10 parts per million. Specifically, a three-dimensional data matrix in CSV format was exported, containing sample information, metabolite names, and mass spectral response intensity. Internal standard peaks, as well as any known false positive peaks (including noise, column bleed, and derivatized reagent peaks), were removed to eliminate redundancy. Metabolites were identified by searching databases, with the main ones being the HMDB (http://www.hmdb.ca/), Metlin (https:/metlin.scripps.edu/), and the Majorbio Database. The data analyses were performed on the majorbio cloud platform (an online analysis platform) (Majorbio Biotech Co., Ltd., Shanghai, China). Metabolites with MS/MS fragment scores above 30 were considered as confidently identified. Initially, the data matrix underwent preprocessing steps. This involved retaining at least 80% of the metabolic features detected in any set of samples. For samples with

metabolite levels below the lower limit of quantification, the minimum metabolite value was estimated and each metabolic signature was normalized to the sum. To mitigate errors from sample preparation and instrument instability, the response intensities of the mass spectrometry peaks in the samples were normalized using the sum normalization method to generate the normalized data matrix. Additionally, variables from QC samples with a relative standard deviation (RSD) greater than 30% were excluded and log10 transformation was applied to the data matrix for subsequent analysis (Fig. S1). Then, we utilized the R package "ropls" (Version 1.6.2) for principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). The model's stability was assessed through a seven-cycle interactive validation, incorporating a Student's *t*-test with a two-tailed test approach. Data conversion for PCA and OPLS-DA was standardized to unit variance to ensure comparability across variables. The confidence level for PCA and OPLS-DA was set at 95%, with OPLS-DA permutations conducted 200 times to validate the model robustness. This was demonstrated in the PCA plot, where the tight clustering of all the QC samples indicated a robust and stable detection system (Fig. S2). Metabolites exhibiting a VIP value greater than 1 and a *P*-value less than 0.05 were identified as significantly different. This determination was based on the VIP values derived from the OPLS-DA model and the *P*-values obtained from the Student's *t*-test. We mapped the differential metabolites among the groups (PEV vs. CON; BPS vs. CON) onto their respective biochemical pathways using metabolic enrichment and pathway analysis, referencing the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Furthermore, we employed Python packages, specifically "scipy.stats" (https://docs.scipy.org/doc/scipy/), to perform enrichment analysis. This analysis facilitated the identification of the most pertinent biological pathways affected by the experimental treatments. We applied the Benjamini-Hochberg (BH) procedure for multiple testing correction and utilized Relative-betweenness Centrality within our Topological Methods to refine our understanding of the data's biological significance.

2.3.4. Meat sample collection and analysis

On d 110, thirty bulls, ten from each treatment group and closest to the average BW, were selected for slaughter. The bulls were fasted for 24 h before slaughter, and meat samples, specifically the longissimus dorsi muscle between ribs 12 and 13, were taken after 48 h of carcass aging and then stored in liquid nitrogen for subsequent analysis. The pH values of the longissimus dorsi muscle at 48 h post-mortem were measured with a portable pH meter (Testo 205, Testo SE & Co. KGaA, Schwarzwald, Germany). The meat color, including redness (a*), yellowness (b*), and lightness (L*), was assessed with a hand-held colorimeter (CR-400, Konica Minolta Investment Ltd, Shanghai, China); chromaticity (C) and hue angle (H) were calculated by using the following formulas:

$$C = \sqrt{\left(a^{*2} + b^{*2}\right)},$$

$$H = a^{*}/b^{*}.$$

Drip loss was determined as described by a previous study (Xu et al., 2020). Briefly, meat samples (2 cm × 3 cm × 5 cm) were weighed, and suspended in a refrigerator at 4 °C for 24 h. Drip loss was calculated by using the following formula:

$$\text{Drop loss (\%)} = (\text{initial weight} - \text{final weight})/\text{initial weight} \times 100.$$

Cooking loss was determined as described previously (Luo and Fan, 2018). Briefly, meat samples (4 cm × 4 cm × 6 cm) were weighed and cooked in a water bath at 80 °C until the center temperature of the meat reached 70 °C. Then, meat samples were cooled to room temperature and re-weighed to calculate cooking loss. Shear force was measured according to the procedures described by Fortin et al. (2005) using a texture analyzer (TMS-PRO, Food Technology Corporation, Virginia, USA). Meat samples (100 g/sample) were processed using a freeze-drying machine (LGJ-25, Sihuan Furui Science and Technology Development Co., Ltd, Beijing, China) to measure moisture (method 950.46), ash (method 920.153), CP (method 992.15), and EE (method 960.39) according to AOAC (2005).

2.3.5. Targeted metabolomic analysis of meat samples

Six biological replicates from each group were used for the LC-MS/MS analysis, conducted on an ExionLC AD system coupled with a QTRAP 6500+ mass spectrometer (Sciex, Framingham, Massachusetts, USA) at Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). In brief, the samples were separated on a HILIC column (Waters UPLC BEH Amide 2.1 × 100 mm, 1.7 μm; Waters Corporation, Milford, Massachusetts, USA) at a constant temperature of 35 °C. The separation process was carried out over a span of 6 min (Table S3). Separation of metabolites was achieved at a flow rate of 1 mL/min with a mobile phase gradient consisting of 95% acetonitrile in water with 0.4% formic acid and 20 mM ammonium formate (solvent A) and 5% acetonitrile in water with 0.4% formic acid and 20 mM ammonium formate (solvent B).

Mass spectrometric data were collected using a UHPLC coupled to a QTRAP 6500+ mass spectrometer equipped with an electrospray ionization (ESI) source operating in positive mode. The parameters were set as follows: source temperature 350 °C; CAD gas pressure medium; both Ion Sources Gas1 and Gas2 at 70 psi; IonSpray Voltage (IS) 5500 V.

The LC-MS raw data were imported into Sciex software OS. All ion fragments were automatically identified and integrated by using default parameters; moreover, all integration was checked manually. The metabolite concentration of samples was calculated according to the linear regression standard curve.

2.4. Data analysis

The growth performance and economic benefits data were analyzed using the MIXED model in SAS 9.4 (SAS Institute, Cary, NC, USA) as follows:

$$Y_{ijk} = \mu + T_i + P_j + T_i \times P_j + S_k + e_{ijk},$$

where Y_{ijk} is the dependent variable; μ is the overall mean; T_i is the fixed effect of the (i)-th treatment; P_j is the fixed effect of the (j)-th period; $T_i \times P_j$ is the interaction effect between the (i)-th treatment and the (j)-th period; S_k is the random effect of the (k)-th bull; e_{ijk} is the random error term. The covariance structure for the random effects and errors was modeled using variance components. If the treatment × period interaction effect was significant, Tukey's multiple comparisons test was applied.

The blood metabolite and meat quality data were analyzed using the MIXED model in SAS 9.4 (SAS Institute, Cary, NC, USA) as follows:

$$Z_{mn} = a + B_m + C_n + d_{mn},$$

where Z_{mn} is the dependent variable; a is the overall mean; B_m is the fixed effect of the (m)-th treatment; C_n is the random effect of the (n)-th bull; d_{mn} is the random error term. The covariance structure for the random effects and errors was modeled using

variance components. If the treatment effect was significant, Tukey's multiple comparison test was applied.

The statistical results are presented in the form of least squares mean (LSM) and standard error of the mean (SEM). A significant difference was declared at $P < 0.05$, a highly significant difference at $P < 0.01$, and a trend was observed at $0.05 < P < 0.10$.

3. Results

3.1. Growth performance and economic benefits

The ADG and feed efficiency were similar across all treatments during the experiment (Table 3). The BPS group showed the lowest DMI among the three treatments ($P < 0.001$). Additionally, the feed cost for the BPS group was lower than the other two groups ($P < 0.001$). However, there were no statistically significant differences in economic indicators such as weight gain income and profit return among the three treatments (Table 3).

3.2. Serum metabolites

As shown in Table 4, dietary treatment had no significant effect on serum energy metabolic indices such as GLU, CHO, TG, HDL-C, and LDL-C throughout the study period ($P > 0.05$).

However, serum amino acid concentrations differed among the three treatments. The serum concentrations of the essential amino acids (EAAs) lysine ($P = 0.003$) and threonine ($P = 0.029$) in the PEV group were higher than those in the CON group, whereas the phenylalanine concentration in the PEV group was lower than that in the CON group ($P = 0.028$). There were more differences observed between the BPS and CON groups: the serum concentrations of three EAAs (lysine, histidine, and threonine) in the BPS group were higher than those in the CON group ($P < 0.05$), and the serum concentrations of arginine and four non-essential amino acids (NEAAs; alanine, glutamic acid, aspartic acid, and glycine) in the BPS group were lower than in the CON group ($P < 0.05$). However, dietary treatment had no significant effect on total essential amino acids (TEAA), total nonessential amino acids (TNEAA), and total amino acids (TAA) ($P > 0.05$).

3.3. Untargeted liquid chromatography–mass spectrometry metabolomics of serum

Collectively, a total of 978 metabolites (512 for positive ion mode and 466 for negative ion mode) were identified. To exclude signals that were not related to model classification and to obtain information on significantly different metabolites between the PEV and CON groups and BPS and CON groups, a supervised model analysis, namely OPLS-DA, was used to analyze the three sample data sets (Fig. 1A and B). The samples were well separated, indicating significant differences in serum metabolites between the PEV and CON groups and BPS and CON groups. The intercept between the Q^2 tropic and Y axis in the corresponding validation chart was less than 0.05 (Fig. 1C and D), which indicated that the models were robust and reliable, and no overfitting occurred.

Based on the above analysis, Student's *t*-tests were conducted on metabolites with a VIP >1 in the OPLS-DA model, using $P < 0.05$ as the judgment criterion, to identify intergroup differential metabolites. A total of 316 different metabolites (upregulated: $n = 229$; downregulated: $n = 87$) were differentially expressed between the PEV and CON groups ($P < 0.05$ and VIP >1; Fig. 2A), and a total of

Table 4
Effects of dietary PEV and BPS on serum metabolite concentrations of bulls.

Item	Treatment			SEM	*P*-value
	CON	PEV	BPS		
Energy metabolites, mmol/L					
Glucose	2.94	2.42	2.14	0.149	0.112
Cholesterol	2.38	2.70	2.52	0.079	0.223
Triglyceride	0.23	0.24	0.24	0.010	0.730
HDL-C	1.43	1.58	1.49	0.035	0.187
LDL-C	0.28	0.35	0.27	0.017	0.102
EAA, μmol/L					
Lysine	81.70^{b}	98.21^{a}	107.26^{a}	3.113	0.003
Methionine	26.21	26.56	23.79	0.824	0.352
Arginine	150.05^{a}	132.13^{a}	105.59^{b}	5.245	0.002
Leucine	175.95	164.78	163.86	2.883	0.188
Isoleucine	99.03	91.50	93.25	1.712	0.179
Valine	221.00	216.77	215.00	4.185	0.856
Histidine	129.05^{b}	138.93^{b}	165.68^{a}	4.618	0.004
Threonine	69.19^{b}	83.24^{a}	87.70^{a}	2.894	0.029
Phenylalanine	54.72^{a}	47.61^{b}	49.84^{ab}	1.132	0.028
Tryptophane	41.43	37.73	39.53	0.823	0.178
NEAA, μmol/L					
Alanine	292.65^{a}	287.63^{a}	234.69^{b}	6.872	<0.001
Glutamic acid	50.12^{a}	46.17^{a}	32.44^{b}	2.089	<0.001
Aspartic acid	11.71^{b}	11.06^{a}	6.88^{b}	0.654	0.004
Glycine	463.41^{a}	455.83^{a}	362.36^{b}	13.303	0.002
Serine	114.40	117.33	107.60	3.343	0.490
Tyrosine	48.39	47.71	52.13	1.301	0.352
Asparagine	34.23	37.96	48.31	2.397	0.057
Glutamine	277.36	283.33	318.73	12.455	0.383
Proline	91.46	96.63	97.46	2.497	0.607
Cysteine	2.51	1.35	0.31	0.387	0.087
TEAA, μmol/L	1048.17	1037.35	1051.37	15.792	0.929
TNEAA, μmol/L	1386.21	1384.99	1260.88	24.632	0.065
TAA, μmol/L	2434.39	2422.35	2312.25	32.627	0.287

CON = control; PEV = peanut vine; BPS = *Broussonetia papyrifera* wrapped silage; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; EAA = essential amino acids; NEAA = nonessential amino acids; TEAA = total essential amino acids; TNEAA = total non-essential amino acids; TAA = total amino acids; SEM = standard error of the mean.
[a,b] Within a row, different superscripts mean significant difference ($P < 0.05$).

Table 3
Effects of PEV and BPS on the growth performance and economic benefits of bulls.

Item	Pre-period (d 0 – d 50)			Post-period (d 50 – d 109)			Whole-period (d 0 – d 109)			SEM	*P*-value		
	CON	PEV	BPS	CON	PEV	BPS	CON	PEV	BPS		Treatment	Time	Time × Treatment
Growth performance													
DMI, kg/d	9.03^{a}	9.25^{a}	8.56^{b}	8.99^{a}	9.23^{a}	8.16^{b}	9.01^{a}	9.24^{a}	8.34^{a}	0.086	<0.001	0.788	0.962
ADG, kg/d	0.81^{y}	0.68^{y}	0.67^{y}	0.86^{x}	0.87^{x}	1.01^{x}	0.84^{xy}	0.78^{xy}	0.86^{xy}	0.027	0.575	0.025	0.552
Feed efficiency	0.09^{y}	0.07^{y}	0.08^{y}	0.10^{x}	0.09^{x}	0.12^{x}	0.09^{xy}	0.08^{xy}	0.10^{xy}	0.003	0.110	0.007	0.449
Economic benefits													
Feed cost, ¥	912.84^{bz}	917.72^{bz}	847.62^{bz}	1072.58^{by}	1080.66^{by}	954.05^{by}	1985.42^{ax}	1998.38^{ax}	1801.67^{ax}	12.384	<0.001	<0.001	0.520
Gain income, ¥	1257.88^{z}	1054.85^{z}	1045.55^{z}	1569.08^{y}	1591.43^{y}	1847.32^{y}	2826.96^{x}	2646.27^{x}	2892.86^{x}	60.993	0.568	<0.001	0.766
Profit return, ¥	345.04^{y}	137.13^{y}	197.92^{y}	496.50^{x}	510.77^{x}	893.27^{x}	841.54^{x}	679.90^{x}	1091.19^{x}	59.117	0.167	<0.001	0.615

CON = control; PEV = peanut vine; BPS = *Broussonetia papyrifera* wrapped silage; DMI = dry matter intake; ADG = average daily gain; SEM = standard error of the mean.
[a,b,c] Within a row, different superscripts indicate a significant difference in treatment effects ($P < 0.05$).
[x,y,z] Within a row, different superscripts indicate a significant difference in time effects ($P < 0.05$).

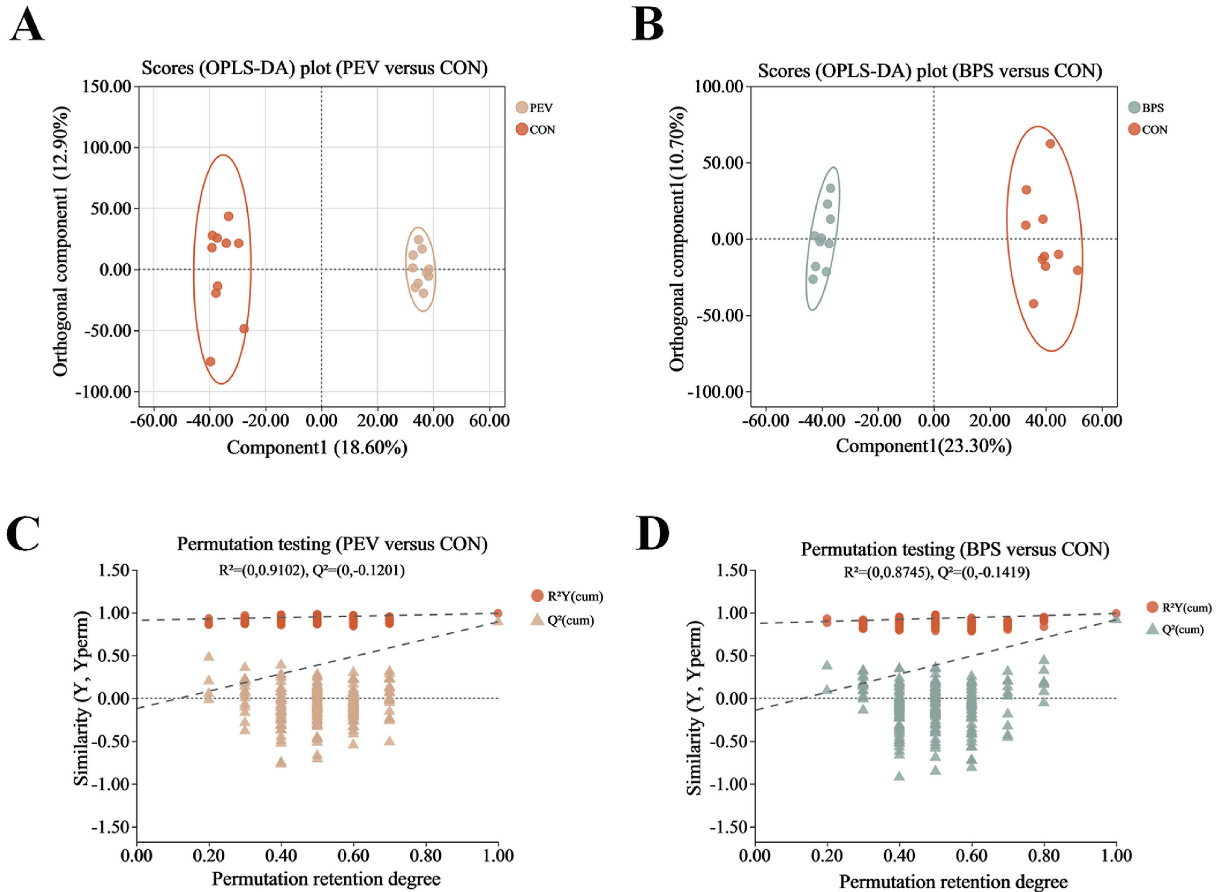

Fig. 1. Comparison analysis of serum metabolites. Orthogonal partial least squares discriminant analysis (OPLS-DA) score plots of serum differential metabolites: PEV vs. CON (A) and BPS vs. CON (B), and OPLS-DA permutation testing plots of serum differential metabolites: PEV vs. CON (C) and BPS vs. CON (D). CON = control; PEV = peanut vine; BPS = *Broussonetia papyrifera* wrapped silage.

434 different metabolites (upregulated: $n = 268$; downregulated: $n = 166$; Fig. 2B) were differentially expressed between the BPS and CON groups ($P < 0.05$ and VIP >1). Subsequently, we identified two sets of differential metabolites (PEV vs. CON, $n = 316$; BPS vs. CON, $n = 434$) for further analysis.

Moreover, the KEGG functional pathway analysis showed that the differential metabolites between the PEV and CON groups and BPS and CON groups were classified into five first-category metabolic pathways. Furthermore, the differential metabolites between PEV and CON were enriched in 26 s-category metabolic pathways, whereas the differential metabolites between BPS and CON were enriched in 28 s-category metabolic pathways (Fig. 3A and B). Despite the large number of differential metabolites and the various types of pathways involved, we chose to focus on amino acid metabolism. As shown in Fig. 4A and B, the KEGG metabolic pathway enrichment analysis of serum differential metabolites indicated that the differential metabolites between the PEV and CON groups were enriched in the phenylalanine, tyrosine and tryptophan biosynthesis pathway ($P = 0.021$) and lysine degradation pathway ($P = 0.042$), whereas the differential metabolites between the BPS and CON groups were enriched in the phenylalanine, tyrosine and tryptophan biosynthesis pathway ($P = 0.004$), lysine degradation pathway ($P = 0.012$), and serotonergic synapse pathway ($P < 0.001$). Subsequently, we identified that four differential metabolites in the PEV and CON comparison and nine differential metabolites in the BPS and CON comparison were associated with these significantly enriched pathways (Table 5 and Table 6, respectively).

3.4. Meat quality

Dietary treatment did not affect the pH, L*, C, drip loss, cooking loss, and chemical composition (moisture, CP, EE, and Ash) of the meat ($P > 0.05$; Table 7). Regarding meat color, a* value ($P = 0.025$) and H value ($P < 0.001$) of the longissimus dorsi muscle in the BPS group were lower than those in the CON and PEV groups; the b* value of the longissimus dorsi muscle in the BPS group was lower than that in the PEV group ($P = 0.024$). Moreover, the shear force in the PEV group was lower than that in the BPS group ($P = 0.014$).

3.5. Liquid chromatography–mass spectrometry metabolomics of meat

Fig. 5 shows the content of essential amino acids in muscles of the three treatments. Lysine content in meat was higher in the BPS group than in the CON group ($P = 0.005$); however, dietary treatment did not affect the other seven EAAs ($P > 0.05$).

4. Discussion

China is facing a shortage of protein feed, especially the most commonly used protein feed soybean meal, the supply of which relies on imports. In 2023, China's soybean import volume reached 99.40 million metric tons (Rouzi, 2024). Therefore, many researchers have attempted to alleviate this problem by using low protein diets and searching for other unconventional protein feeds and have achieved significant results (Cruz et al., 2018; Freitas et al.,

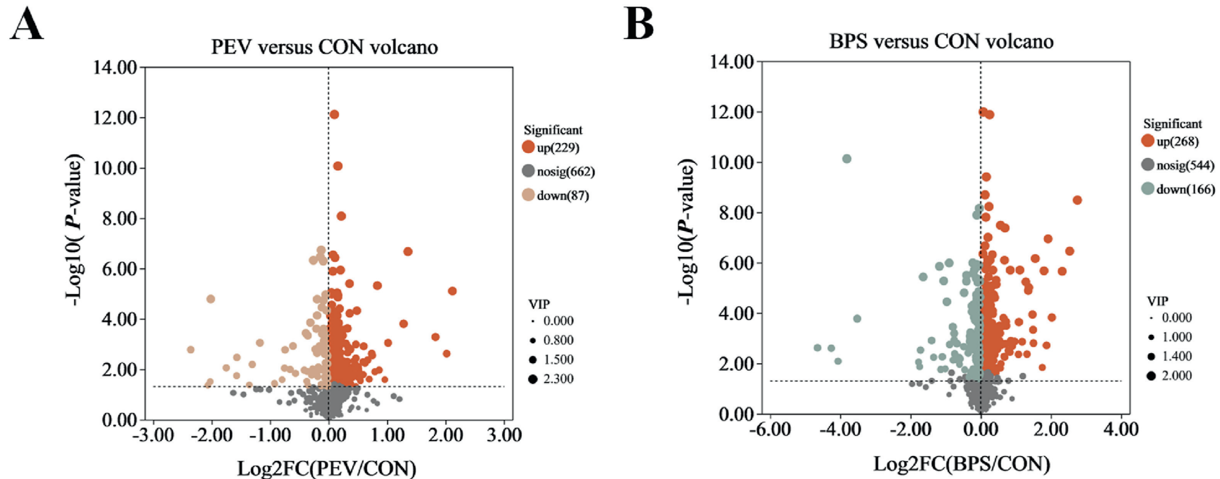

Fig. 2. Volcano plots of serum differential metabolites: PEV vs. CON (A), BPS vs. CON (B). CON = control; PEV = peanut vine; BPS = *Broussonetia papyrifera* wrapped silage; VIP = variable importance in the projection.

2017; Wang et al., 2018; Zhou et al., 2022). However, few studies have used roughage sources to reduce the use of soybean meal. In the present study, we chose two types of roughage sources with high CP content in an attempt to reduce the use of soybean meal. We found that the CP content of PEV was 7.35%, and the CP content of BPS was 14.71%. The CP content of PEV was lower than that reported in previous studies, which might be related to variety, harvesting time, and treatment process (Zhang et al., 2022). However, the protein content of BPS was similar to that reported previously (He et al., 2021). Accordingly, the decrease in the use of soybean meal was more significant in the BPS group (29.41% decrease) than in the PEV group (23.53% decrease).

To maintain health and productivity, animals require a balanced diet with reasonable amounts of various nutrients (Ganguly et al., 2021). Therefore, we attempted to design three iso-nitrogen, iso-energy dietary formulas based on existing feed ingredients. However, several dietary parameters, such as DM content, and the ratio of each nutrient affect growth performance (Semba et al., 2016), and therefore the same growth performance cannot be achieved with different dietary treatments. Nutrient intake is one of the most important factors determining animal performance (Zhang et al., 2022). In the present study, the BPS group showed the lowest DMI throughout the experimental period. DMI of animals is regulated by the negative feedback of satiety (Han et al., 2021), and studies have shown that DMI is influenced by multiple factors such as feed palatability, nutrient levels, fiber content, and DM content (Ding and Meng, 2013). Considering that the nutrient levels (energy and CP) and fiber content of the three diets were comparable, we speculated that the low DMI of BPS was due to high water content and poor palatability. In general, the higher the moisture content of the feed, the lower the feed intake of animals (Kronqvist et al., 2021). Furthermore, owing to the high tannin content and complex protein molecular structure of paper mulberry leaves, the palatability of the BPS feed was low. Silage improves feed palatability to some extent, but it may not be enough to completely eliminate the problem of poor palatability (He et al., 2021). Previous studies have shown that lower quantities of BPS did not significantly affect the ADG of beef cattle (Chen et al., 2021; Hu et al., 2022). However, it has been reported that feeding 5% BPS to beef cattle at the same growth stage increased DMI, which is inconsistent with the present findings; the discrepancy may be related to the difference in cattle breed (Simmental crossbred cattle vs Wuchuan cattle) (Tao et al., 2020). The growth performance of the PEV group was similar to that of the CON group, which is consistent with previous findings (Zhang et al., 2022). According to Chinese dietary beliefs, various parts of cattle such as the rumen, reticulum, omasum, and head hold significant economic value beyond just meat (Du et al., 2023). In order to accurately assess profits, it is more appropriate to consider the live weight of beef cattle. Our analysis revealed that the notable reduction in feed costs, attributed to the lower price of BPS, resulted in cost savings, despite no significant difference in final income. Due to the lower feed intake of BPS in comparison to the other two groups, it is reasonable for the feed cost of BPS to be lower when the feed unit price is similar.

Blood biochemical indicators signify the health status and metabolic level of animal body (Ma et al., 2015). Blood sugar and blood lipids are the main energy substrates of the body and determine the energy supply state and normal metabolic level of the body (Han et al., 2022; Pan et al., 2022). The use of PEV and BPS as substitutes for rice straw did not significantly change the blood sugar (glucose) and blood lipid (CHO, TG, HDL-C, and LDL-C) content of fattening cattle, and both remained within the normal reference range. This finding is consistent with previously reported results (Tao et al., 2020; Xue et al., 2019). Blood amino acid concentration comprehensively reflects the small intestinal absorption and metabolism of amino acids (Brown and Cline, 1974; Eggum, 1970). Consistent with our expectations, dietary changes led to variations in serum amino acid levels in bulls. To obtain useful information on changes in serum amino acid metabolism, we used metabolomics techniques. The results showed that there were differences in metabolites related to serum amino acids among the three treatment groups. L-tryptophan and 3-dehydroquinate are key metabolites involved in phenylalanine, tyrosine, and tryptophan biosynthesis. Upon chemical analysis of TMR, it was observed that the amino acid composition was comparable. The observed upregulation of 3-Dehydroquinate and L-tryptophan may have been influenced by other factors. It is hypothesized that during the silage process, the substantial sugars present in the paper mulberry undergo anaerobic homolactic fermentation by microorganisms, particularly lactic acid bacteria (Li et al., 2021). This process leads to the generation of numerous intermediate products such as fructose-1,6-phosphate and phosphoenolpyruvate, which serve as precursors for 3-3-Dehydroquinate (Zhou et al., 2021). Meanwhile, 3-Dehydroquinate is the precursor of quinate, which acts as a precursor of the shikimate pathway. This pathway is integral to the biosynthesis of aromatic amino acids, including tryptophan

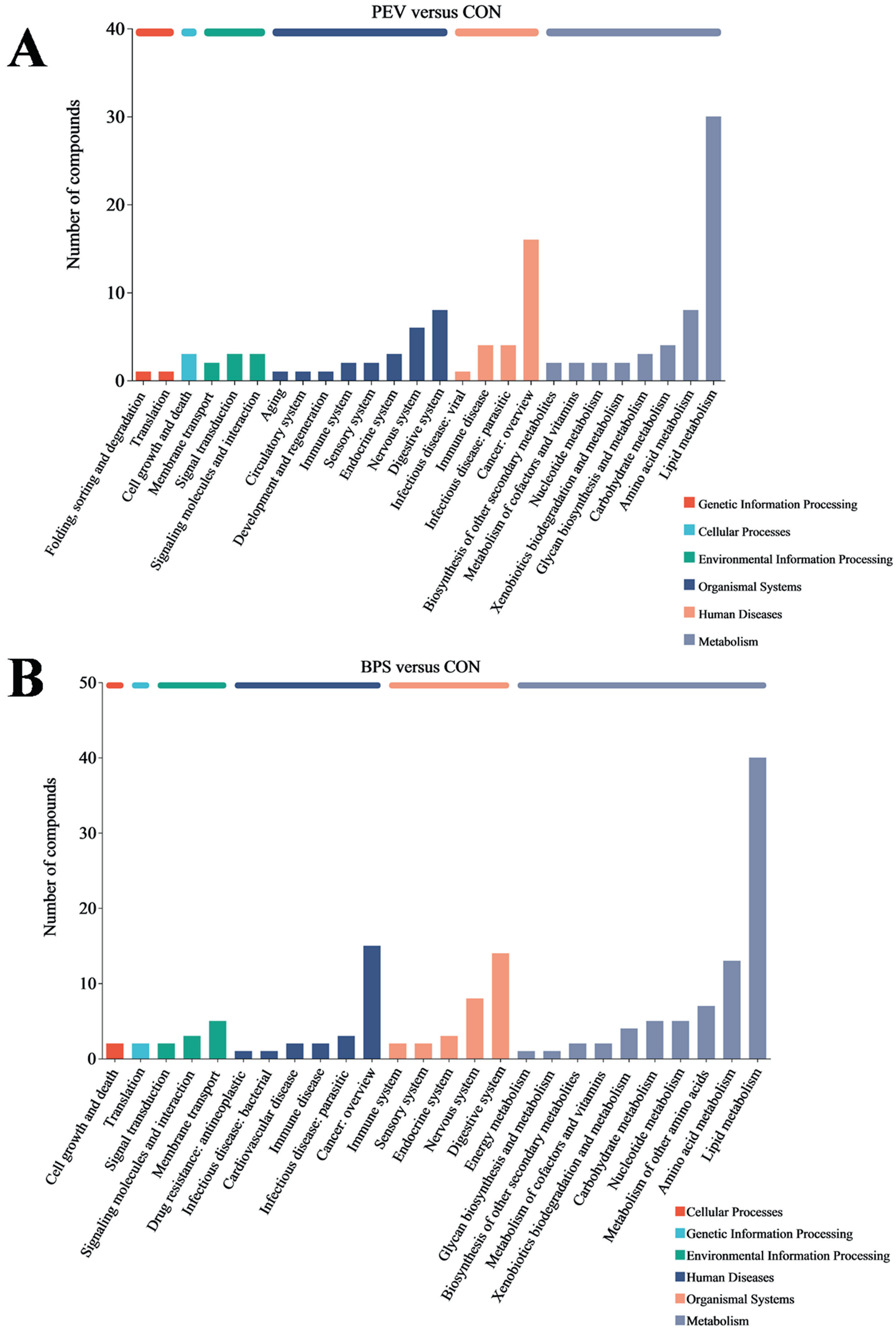

Fig. 3. KEGG functional pathway analysis of serum differential metabolites between the PEV and CON groups (A) and BPS and CON groups (B). CON = control; PEV = peanut vine; BPS = *Broussonetia papyrifera* wrapped silage.

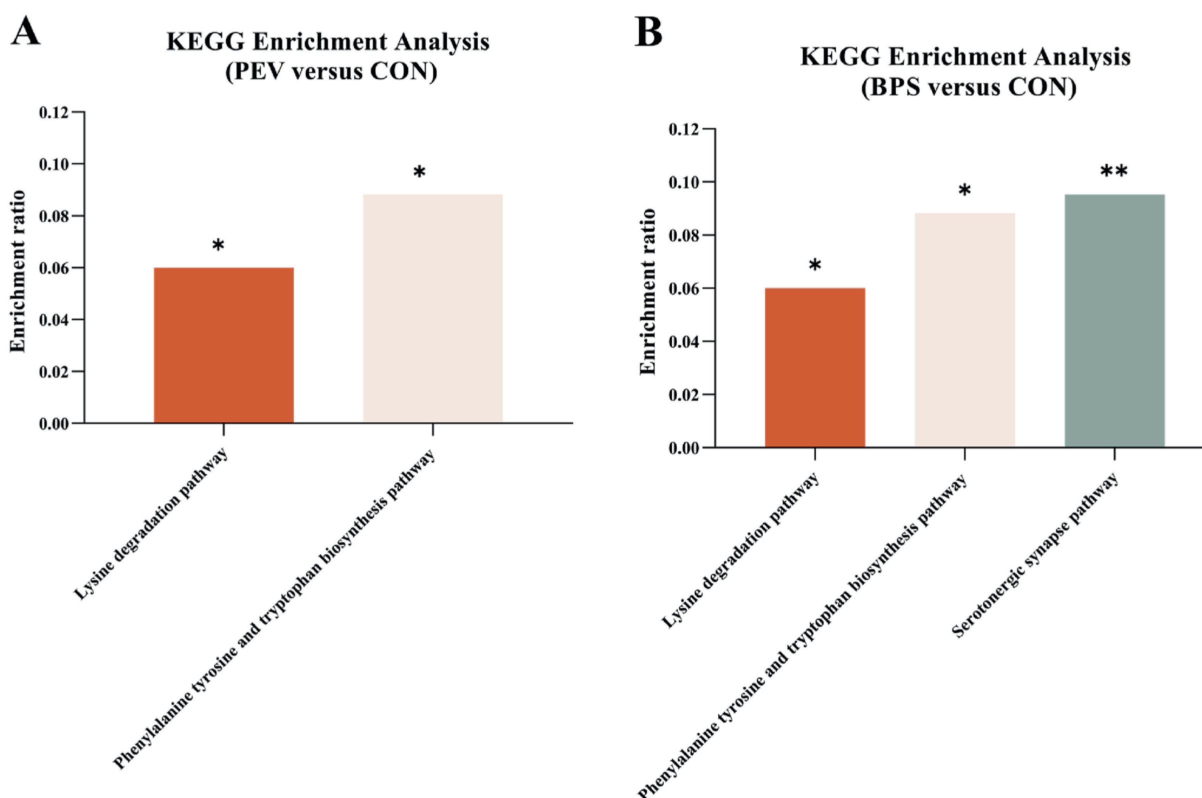

Fig. 4. KEGG enrichment analysis of serum differential metabolites between the PEV and CON groups (A) and BPS and CON groups (B). CON = control; PEV = peanut vine; BPS = *Broussonetia papyrifera* wrapped silage. *$P < 0.05$, **$P < 0.01$.

Table 5
Identification of serum differential metabolites between the PEV and CON groups ($P < 0.05$ and VIP >1; four species) associated with amino acid metabolic pathways.

Metabolite	VIP_pred_OPLS-DA	FC (PEV/CON)	Direction of regulation	*P*-value
Phenylalanine, tyrosine and tryptophan biosynthesis				
L-Tryptophan	1.2171	1.3351	Up	0.017
3-Dehydroquinate	1.8575	1.1172	Up	<0.001
Lysine degradation				
5-Aminopentanamide	1.8431	1.0506	Up	<0.001
N6-Acetyl-L-lysine	1.3083	1.4065	Up	0.008

CON = control; PEV = peanut vine; VIP_pred_OPLS-DA = variable importance in OPLS-DA; FC (PEV/CON) = fold change ratio (PEV/CON).

Table 6
Identification of serum differential metabolites between the BPS and CON groups ($P < 0.05$ and VIP >1; nine species) associated with amino acid metabolic pathways.

Metabolite	VIP_pred_OPLS-DA	FC (BPS/CON)	Direction of regulation	*P*-value
Phenylalanine, tyrosine and tryptophan biosynthesis				
L-Tryptophan	1.2529	1.3966	Up	0.003
3-Dehydroquinate	1.0857	1.0620	Up	0.018
Lysine degradation				
5-Aminopentanamide	1.6431	1.0442	Up	<0.001
5-Aminopentanoic acid	1.0497	0.9697	Down	0.024
Saccharopine	1.1657	1.2863	Up	0.014
Serotonergic synapse				
5-Hydroxyindoleacetic acid	1.2086	0.9086	Down	0.005
TXB2	1.4862	0.8641	Down	<0.001
Thromboxane B2	1.6603	0.5123	Down	<0.001
Arachidonic acid	1.0118	0.8025	Down	0.031

CON = control; BPS = *Broussonetia papyrifera* wrapped silage; VIP_pred_OPLS-DA = variable importance in OPLS-DA; FC (BPS/CON) = fold change ratio (BPS/CON).

(Gallego-Giraldo et al., 2011). In the present study, we also found that tryptophan was upregulated. Tryptophan is an essential amino acid necessary for protein synthesis (Jiang et al., 2022). Furthermore, tryptophan is hydroxylated to form 5-hydroxytryptamine and further converted into serotonin (Mora-Villalobos and Zeng, 2018); the serotonin receptor subtype 5-hydroxytryptamine 2C (5-HT2cR) decreases satiety and food intake via the hypothalamic melanocortin system (Xu et al., 2008). Our analysis of the

Table 7
Effects of dietary PEV and BPS on the quality of meat (longissimus dorsi) of Simmental crossbred bulls.

Item	Treatment			SEM	P-value
	CON	PEV	BPS		
pH	5.46	5.59	5.49	0.052	0.567
Meat color					
L*	36.40	34.48	36.26	0.765	0.536
a*	18.78[a]	17.66[a]	14.25[b]	0.731	0.025
b*	8.46[ab]	6.95[b]	9.15[a]	0.346	0.024
C	20.67	19.00	17.07	0.686	0.097
H	2.27[a]	2.67[a]	1.58[b]	0.120	<0.001
Drip loss,%	13.24	13.73	12.39	0.644	0.707
Cooking loss,%	31.43	30.63	33.04	0.666	0.335
Shear force, N	34.74[ab]	27.38[b]	45.95[a]	2.718	0.014
Chemical composition (%, DM basis)					
Moisture	73.06	72.02	72.71	0.357	0.498
Crude protein	80.92	77.55	78.62	0.999	0.384
Ether extract	10.24	13.01	11.88	1.088	0.595
Ash	6.81	6.57	6.66	0.226	0.914

CON = control; PEV = peanut vine; BPS = *Broussonetia papyrifera* wrapped silage; L* = lightness; a* = redness; b* = yellowness; C = chromatic value; H = hue angle value; SEM, standard error of the mean.
[a,b] Within a row, different superscripts mean significant difference ($P < 0.05$).

serotonergic synapse pathway found that 5-hydroxytryptamine led to the downregulation of arachidonic acid through the 5-HT2cR/Gq/PLA2 signaling pathway. Arachidonic acid is the backbone of both endocannabinoids 2-arachidonoylglycerol and anandamide; studies have shown that an increase in arachidonic acid in the diet leads to an increase in 2-arachidonoylglycerol and anandamide, while also increasing animal feed intake (Alvheim et al., 2012). The upregulation of tryptophan and downregulation of arachidonic acid may be the key reasons for the decrease in DMI in the BPS group. The lysine content in TMR of the three treatment groups is similar, and it is acknowledged that the bulls themselves cannot synthesize lysine (Liu et al., 2019). However, the BPS group showed significantly increased serum and muscle lysine levels compared to the CON group, suggesting a potential increase in lysine synthesis by rumen microorganisms. The glycolysis process of silage feed involves the conversion of phosphofructose 6-phosphate to pyruvic acid phosphate, and enolase dehydrates 2-phosphoglycerate to form phosphoenolpyruvate (Narita et al., 2020), providing substrates for the main lysine synthesis pathway of the rumen microbiota, known as the diaminopimelic acid pathway, which promotes the synthesis of rumen amino acids (Bukhari and Taylor, 1971; Zabriskie and Jackson, 2000). Furthermore, the rumen also contains the α-aminoadipic acid pathway, another lysine synthesis pathway, allowing certain fungi and bacteria to utilize saccharopine to synthesize lysine through saccharopine dehydrogenase (Kosuge and Hoshino, 1998). Our results indicated an increase in serum saccharopine in the BPS group, suggesting that rumen microorganisms may synthesize lysine through this pathway. In addition, 5-aminopentamide is the primary metabolite of lysine, which is further degraded into 5-aminopentanoic acid, which is degraded into glutarate and then converted into acetyl-CoA, which participates in the citrate cycle (Thompson et al., 2019). The upregulation of 5-aminopentamide and the downregulation of 5-aminopentanoic acid in the BPS group seem to be contradictory; therefore, we speculate that the conversion of 5-aminopentamide to 5-aminopentanoic acid was inhibited, which facilitated the participation of lysine, along with other amino acids, in protein synthesis and deposition in the body tissue.

The majority of meat quality characteristics, including meat color, tenderness, water holding capacity, and other muscle characteristics, are strongly linked with muscle pH (El et al., 2004). Meat

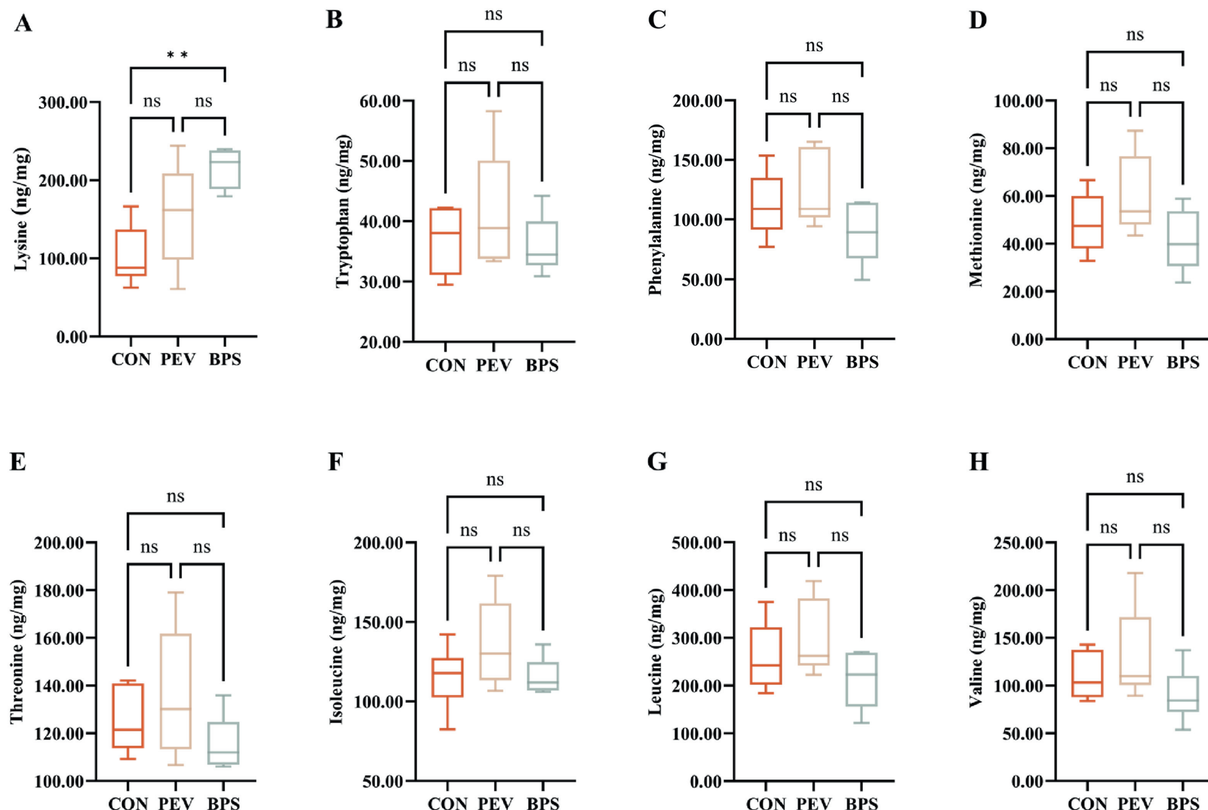

Fig. 5. Levels of essential amino acids in the longissimus dorsi muscle. (A) Lysine; (B) Tryptophan; (C) Phenylalanine; (D) Methionine; (E) Threonine; (F) Isoleucine; (G) Leucine; (H) Valine. CON = control; PEV = peanut vine; BPS = *Broussonetia papyrifera* wrapped silage. **$P < 0.01$.

color is considered an important indicator of meat quality for consumers, and it influences consumers' attitudes and purchasing intent (Grunert et al., 2004). The lightness value is related to the muscle hydraulic power and tenderness (Martins et al., 2014). The redness value is closely related to the oxygenation of myoglobin; muscle tissue with low oxygen-carrying capacity appears purple red, whereas that with high oxygen-carrying capacity appears bright red (Bekhit and Faustman, 2005; Qiao et al., 2022). The yellowness value is a measure of yellowness (Ball et al., 2021). In general, the higher the a* value and the lower the b* value, the higher the H value and the better the meat quality (Hughes et al., 2020). The shear force is used as the index to determine meat tenderness (Zhang et al., 2021). Meat in the PEV group showed not only the highest H value but also the lowest shear force, whereas the BPS group showed the opposite trend, indicating that PEV supplementation improves meat quality. Previous studies have reported that BPS improves meat quality (Hu et al., 2022; Tao et al., 2020); however, there has been little research on the use of BPS in beef cattle, and several factors affect meat quality. Therefore, further research and more data are needed to verify the present findings. In addition, the type and relative content of amino acids in beef determine the nutritional value and flavor of beef, which constitute an important index for evaluating meat quality (Yang et al., 2022). Excluding an increase in lysine content in muscle in the BPS group, the dietary treatments had no significant effect on the levels of other EAAs. This is consistent with our previous speculation that the upregulated lysine in the BPS group, along with other amino acids, participates in protein synthesis and deposition in body tissues. As the first limiting amino acid, lysine is believed to promote muscle fiber hypertrophy by increasing protein synthesis (Jin et al., 2019). Our research using two types of roughages to reduce soybean meal use shows this is feasible. The substitution of PEV did not adversely affect production performance, but improved beef quality compared with BPS. The replacement of BPS reduced DMI, but did not affect its growth performance and increased the lysine content in beef. However, we have to admit that due to limited experimental conditions, we only used 5% of the alternative treatment. In the future, experiments should be conducted on a larger scale to determine the optimal addition amounts of the two types of roughages. Although the experimental treatment only made minor adjustments to TMR, we observed significant reductions in feed intake and feed cost in the BPS group. This treatment also impacted the subsequent amino acid metabolism process, resulting in changes in the lysine content in muscle tissue. Notably, due to the fundamental similarities between PEV and rice straw, we found that the outcomes for PEV were comparable to those of the CON group. These findings highlight the importance of considering different unconventional roughage sources and even slight modifications to feed formulations, as they can influence the growth performance of beef cattle.

5. Conclusion

Substituting rice straw with PEV and BPS in beef cattle feed reduced the use of soybean meal but had no adverse effects on growth performance. The addition of BPS affected the amino acid metabolism of bulls, thus decreasing feed intake and increasing the content of lysine in meat. Nevertheless, the quality of meat in the PEV group was better than that in the BPS group.

CRediT authorship contribution statement

Xin Yi: Writing – original draft, Visualization. **Yueming Li:** Validation, Software, Data curation. **Yue Liu:** Writing – review & editing. **Minzhe Zhang:** Resources. **Zhenming Zhou:** Project administration. **Qingxiang Meng:** Supervision. **Hao Wu:** Writing – review & editing, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.11.013.

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