Research Article

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Balanced branched-chain amino acids modulate meat quality by adjusting muscle fiber type conversion and intramuscular fat deposition in finishing pigs

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Abstract

BACKGROUND: Pork is an important food for humans and improving the quality of pork is closely related to human health. This study was designed to investigate the effects of balanced branched-chain amino acid (BCAA)-supplemented protein-restricted diets on meat quality, muscle fiber types, and intramuscular fat (IMF) in finishing pigs.

RESULTS: The results showed that, compared with the normal protein diet (160 g kg^{-1} crude protein), the reduced-protein diet (120 g kg^{-1} crude protein) supplemented with BCAAs to the ratio of 2:1:2 not only had higher average daily gain ($P < 0.05$) and carcass weight ($P < 0.05$) but also improved meat tenderness and juiciness by decreasing shear force ($P < 0.05$) and increasing water-holding capacity ($P < 0.05$). In particular, this treatment showed higher ($P < 0.05$) levels of phospho-acetyl-CoA carboxylase (P-ACC) and peroxisome proliferation-activated receptor-γ (PPARγ), and lower ($P < 0.05$) levels of P-adenosine 5'-monophosphate (AMP)-activated protein kinase (P-AMPK), increasing the composition of IMF and *MyHC I* ($P < 0.05$) in the *longissimus dorsi* muscle (LDM). In terms of health, this group increased eicosapentaenoic acid (EPA) ($P < 0.01$) and desirable hypocholesterolemic fatty acids (DHFA) ($P < 0.05$), and decreased atherogenicity (AI) ($P < 0.01$) and hypercholesterolemic saturated fatty acids (HSFA) ($P < 0.05$).

CONCLUSION: Our findings suggest a novel role for a balanced BCAA-supplemented restricted protein (RP) diet in the epigenetic regulation of more tender and healthier pork by increasing IMF deposition and fiber type conversion, providing a cross-regulatory molecular basis for revealing the nutritional regulation network of meat quality.
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Keywords: branched-chain amino acid ratios; meat quality; intramuscular fat; muscle fiber type; finishing pigs

INTRODUCTION

Pork occupies a dominant position in the world's consumer market, and its quality is closely related to human health.[1] In animal husbandry, fat deposition is closely related to meat quality, especially intramuscular fat (IMF) deposition,[2] as delicious and juicy meat requires a moderate fat content in the muscles to satisfy consumers.[3] Controlling the composition of fatty acids, which produce flavors in IMF, such as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA)[4] plays an important role in improving meat quality. On the other hand, a large body of literature suggests that adjusting the conversion of muscle fiber types through nutritional manipulation is also likely to improve pork quality.[5–7] In fact, adult skeletal muscles display plasticity and can switch between different fiber types in response to changes in nutritional intake: myosin heavy chain (MyHC) I (slow oxidation type) ⇌ MyHC IIa (fast oxidation type) ⇌ MyHC IIx (intermediate type) ⇌ MyHC IIb (fast glycolysis type), significantly affecting meat color, pH, water-holding capacity (WHC), tenderness, juiciness, and flavor.[8] Generally, the meat quality increases when the proportion of oxidation fibers increase, and it decreases when the proportion of glycolysis fibers increase.[5]

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Branched-chain amino acids (BCAAs), including leucine (Leu), isoleucine (Ile), and valine (Val), are mainly distributed in muscle tissue and account for the majority of the essential amino acids found in muscles.[5] The research on BCAAs in pigs therefore mainly focuses on meat quality, especially IMF deposition and muscle fiber conversion. For example, the supplementation low-protein diet with Leu was found to increase the drip loss significantly, and the IMF content of finishing pig models,[9] indicating the potential effect of Leu on improving pork quality. Supplementation of Ile in finishing pig diets inhibited the adenosine 5′-monophosphate (AMP)-activated protein kinase (AMPK)-acetyl-CoA carboxylate (ACC)-signaling pathway, and promoted the deposition of IMF.[10] It has also been reported that Leu promotes the differentiation of porcine skeletal muscle satellite cells to form slow muscle fibers by activating the silent information regulator of the transcription 1 (SIRT1)/AMPK signaling pathway.[11] Both the dietary ratio of BCAAs and whether the balanced BCAAs can modulate the muscle fiber traits and IMF deposition therefore need to be managed closely.

In recent years it has been reported that decreasing dietary crude protein (CP) can hinder performance while increasing lipid metabolism in pigs, thereby affecting the quality of pork.[12] It has been estimated that limiting CP content may not provide sufficient essential amino acids (AAs) to maintain nutritional requirements. In addition to the studies mentioned above,[6,7,9,10] which provide a basis for BCAAs to improve meat quality from the perspective of muscle fiber type conversion and IMF deposition, we have found that dietary balanced BCAA ratios may regulate muscle fiber types in growing pigs.[5] We also found that leucine plays important roles in metabolic homeostasis. In this study, we would like to deeply determine that when the supplementation of Leu is sufficient , whether the increase of the other two BCAAs (Ile or Val) will regulate the metabolism, meaning the importance of the balance of BCAAs. The effects and potential mechanisms of five treatments, namely a normal protein (NP diet (160 g kg^{-1} CP), a restricted protein (RP) diet (120 g kg^{-1} CP), and RP diets supplemented with BCAAs to three ratios (Leu/Ile/Val = 2:1:1, 2:2:1,

Table 1. Composition of the experimental diets (as-fed basis)

			RP		
Item[a]	NP	RP	BCAA2:1:1	BCAA2:2:1	BCAA2:1:2
Ingredient (g kg^{-1} as fed)					
Corn	668.8	808.4	811.8	811.8	811.8
Soybean meal	239.0	117.5	98.0	98.0	98.0
Wheat bran	60.0	30.0	30.0	30.0	30.0
Soybean oil	8.8	14.4	22.0	22.0	22.0
Lysine	—	3.7	4.2	4.2	4.2
Methionine	—	0.3	0.5	0.5	0.5
Threonine	—	1.1	1.3	1.3	1.3
Tryptophan	—	0.3	0.3	0.3	0.3
Leucine	—	—	2.9	0.2	0.2
Isoleucine	—	—	2.6	6.5	1.4
Valine	—	—	2.0	0.8	5.9
CaHPO4	5.0	6.0	6.0	6.0	6.0
Limestone	5.4	5.3	5.4	5.3	5.4
Salt	3.0	3.0	3.0	3.0	3.0
Premix[b]	10.0	10.0	10.0	10.0	10.0
Nutrient content (g kg^{-1} dry matter)					
Digestible energy (MJ kg^{-1})	14.21	14.23	14.22	14.22	14.22
Crude protein[c]	160.4	120.3	120.5	120.9	120.7
Total Lys	8.72	8.28	8.11	8.12	8.10
Total Met + Cys	5.57	4.48	4.41	4.43	4.42
Total Thr	6.79	5.81	5.66	5.64	5.63
Total Trp	2.16	1.64	1.50	1.52	1.51
Total Leu	7.12	4.89	7.05	10.87	5.87
Total Ile	14.46	11.57	13.81	11.17	11.18
Total Val	7.36	5.30	5.89	5.71	10.71
Total Ca	5.1	5.0	5.0	5.0	5.0
Total P	4.5	4.0	3.9	3.9	3.9
Available P	2.0	2.0	1.9	1.9	1.9

[a] Diet treatment: NP, 160 g kg^{-1} crude protein level diet (NRC, 2012)[13]; RP, 120 g kg^{-1} crude protein level diet; BCAA2:1:1, RP diet supplemented with BCAAs to the ratio (Leu:Ile:Val) 2:1:1; BCAA2:2:1, RP diet supplemented with BCAAs to the ratio 2:2:1; BCAA2:1:2, RP diet supplemented with BCAAs to the ratio 2:1:2.
[b] Supplied per kg of diet: vitamin A, 10800 IU; vitamin D3, 4000 IU; vitamin E, 40 IU; vitamin K3, 4 mg; vitamin B1, 6 mg; vitamin B2, 12 mg; vitamin B6, 6 mg; vitamin B12, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg; Cu (as copper sulfate), 150 mg; Fe (as ferrous sulfate), 100 mg; Mn (as manganese oxide), 40 mg; Zn (as zinc oxide), 100 mg; I (as potassium iodide), 0.5 mg; and Se (as sodium selenite), 0.3 mg.
[c] Analyzed crude protein and total amino acid content in the diets.

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2:1:2), on meat quality in finishing pigs were studied to determine whether RP diets supplemented with balanced BCAAs could improve meat quality, while reversing the weak performance caused by RP diets. We hypothesized that supplementing diets with balanced BCAAs could increase the IMF content, thereby enhancing meat tenderness and juiciness, and even increasing the composition of healthy fatty acids and oxidative muscle fibers, consequently improving meat quality.

MATERIALS AND METHODS

Animals, diets, and sample collection

All animal procedures were approved by the agriculture animal care and use committee of the Institute of Subtropical Agriculture, Chinese Academy of Sciences, and adhered to the Chinese guidelines on experimental protocols and animal welfare. One hundred and sixty male finishing pigs (Landrace × Large White) with average bodyweight of 59.11 ± 0.46 kg (mean ± SEM) were randomly allotted to one of five dietary treatments with eight replicate pens per treatment and four pigs per pen: a control group in which pigs received a basal diet according to the NRC 2012[13] (160 g kg^{-1} CP, NP), an RP group (120 g kg^{-1}CP) and three RP groups supplemented with different ratios of BCAAs (Leu:Ile:Val = 2:1:1, 2:2:1, 2:1:2) (Table 1). Each replicate was fed in independent pens equipped with four-hole self-feeders and a nipple drinker. The pigs were allowed to consume feed and water freely throughout the experiment. The experiment had duration of 43 days until the average final bodyweight of finishing pigs was 95 kg.

At the end of the trial, pigs close to the average final bodyweight of each pen were selected and transported to a local abattoir before sunrise that day. After at least 10 h of rest, the pigs were electrically stunned, exsanguinated and eviscerated according to the standard commercial procedures. The carcasses were split at the center of the vertebral column. From the right half of each carcass, about 5 g of the *longissimus dorsi muscle* (LDM) between the sixth and seventh ribs was sampled and placed in RNase-free tubes and immediately frozen in liquid nitrogenere for analysis of mRNA expression. Then, 20 g of the LDM was harvested, trimmed for any extra-muscular fat and connective tissues, and stored at −20 °C for IMF content measurement. Subsequently, at 24 h postmortem, the LDM on the left half of each carcass between the sixth and seventh ribs was sampled for the analysis of meat quality characteristics. For HE staining, the LDM between the sixth and seventh ribs was cut to 1 mm^3 and immediately stored in formalin solution.

Amino acid content analysis in the diets

A 0.1 g sample was accurately weighed in the hydrolytic tube, together with 10 mL 6 mol L^{-1} HCl. After the alcohol spray lamp seal, the hydrolytic tubes were placed in a 110° thermostatic tank. After 22 h of hydrolysis, the hydrolytic tubes were removed to cool. The hydrolysate was filtered, rinsed with deionized water several times, and transferred to a 50 mL volumetric flask. One milliliter of filter fluid was dissolved with 1 mL 0.01 mol L^{-1} HCl. The mixtures were centrifuged at 15 000×*g* for 15 min and detected by amino acid analyzer (Hitachi, L8900, Japan).

The crude protein content was measured according to the Association of Analytical Chemists methods.[14] Briefly, a 0.2 g sample was weighed accurately in the tapered bottle, together with 8 mL hydrochloric acid, and this was left at room temperature overnight. Then the tapered bottles were placed on a muffle until the liquid turned transparent. The digested samples were moved to a 100 mL volumetric flask. After cooling, they were diluted with double steaming water to volume, and mixed. Then 8 mL of the mixture was transferred to a 10 mL centrifuge tube and placed on the flow injection analyzer (SEAL, Germany) to be detected.

Meat quality assessment

After slaughtering, the left sides of the carcasses were subjected to measurements of carcass traits and meat quality at the sixth

Table 2. Real-Time PCR Primer Sequence

Gene	bp	Forward	Reverse
ACC	169	5′-AGCAAGGTCGAGACCGAAAG-3′	5′-TAAGACCACCGGCGGATAGA −3′
AMPKα	105	5′- GCATAGTTGGGTGAGCCACA −3′	5′- CCTGCTTGATGCACACATGA −3′
ATGL	80	5′- CAACGCCAAGCACATCTACG −3′	5′- CCAGTATCACCCAGGCAGAC- 3′
CPT1	170	5′- GACAAGTCCTTCACCCTCATCGC -3′	5′- GGGTTTGGTTTGCCCAGACAG −3′
FABP4	227	5′- CAGGAAAGTCAAGAGCACCA −3′	5′- TCGGGACAATACATCCAACA −3′
FATP1	78	5′- ACCACTCCTACCGCATGCAG −3′	5′- CCACGATGTTCCCTGCCGAGT −3′
GAPDH	123	5′-CAAAGTGGACATTGTCGCCATCA-3′	5′- AGCTTCCCATTCTCAGCCTTGACT-3′
HSL	167	5′- CACAAGGGCTGCTTCTACGG −3′	5′- AAGCGGCCACTGGTGAAGAG −3′
LPL	148	5′- CTCGTGCTCAGATGCCCTAC -3′	5′- GGCAGGGTGAAAGGGATGTT −3′
MyHC I	63	5′- GGCCCCTTCCAGCTTGA −3′	5′- TGGCTGCGCCTTGGTTT −3′
MyHC IIa	109	5′- TTAAAAAGCTCCAAGAACTGTTTCA −3′	5′- CCATTTCCTGGTCGGAACTC -3′
MyHC IIb	83	5′- CACTTTAAGTAGTTGTCTGCCTTGAG-3′	5′-GGCAGCAGGGCACTAGATGT −3′
MyHC IIx	79	5′- AGCTTCAAGTTCTGCCCCACT −3′	5′- GGCTGCGGGTTATTGATGG −3′
PPARγ	124	5′-CCAGCATTTCCACTCCACACTA-3′	5′- GACACAGGCTCCACTTTGATG-3′
SREBP1c	218	5′- GCGACGGTGCCTCTGGTAGT −3′	5′- CGCAAGACGGCGGATTTA −3′
UCP3	152	5′- TGCTGGGCACCATTCTCACC−3′	5′- CGATCCCTTGGGCGTGTAAAG −3′

ACC, acetyl-CoA carboxylase; AMPKα, Adenosine 5′-monophosphate (AMP)-activated protein kinase alpha; ATGL, adipose tissue triglyceride lipase; CPT1, carnitine palmitoyl transferase 1; FABP4, fatty acid binding protein 4; FATP1, fatty acid transport protein 1; HSL, hormone-sensitive triglyceride lipase; LPL, lipoprotein lipase; MyHC, myosin heavy chain; PPARγ, peroxisome proliferation-activated receptor-γ; SREPB1c, sterol regulatory element-binding protein 1; UCP3, uncoupling protein type 3.

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to seventh rib. The pH value, represented for meat acidity, was measured 45 min and 24 h postmortem using a pH meter (Matthaus pH Star, Po tmes, Germany). Each sample was replicated in triplicate. Meat color was evaluated in triplicate using a tristimulus colorimeter (Minolta Chroma Meter Measuring Head CR-410 Minolta, Osaka, Japan) to obtain the ⊿L* (lightness), ⊿a* (redness) and ⊿b* (yellowness) values. Before measurement, the tristimulus colorimeter was calibrated using a white tile. The shear force (N) of the LDM was performed as described by Starkey *et al.*[15] with some modifications. Each muscle sample was cooked in a water bath at 70 °C for 20 min. After cooling, the sample was cut into cuboids (2 × 1 × 1 cm) in the direction of the myofibrils. The shear force of the cuboid was measured vertically using a GR-150 Warner–Bratzler shear machine (Shakopee, MN, USA), and the maximal force was recorded. Each sample was replicated eight times. The average of eight cuboids was calculated to determine the shear force value per sample. The cooking loss was determined as follows: a thickness of 2 cm was weighed, then cooked to an internal temperature of 70 °C in a 75 °C thermostatic water bath, cooled to room temperature, wiped with absorbent paper to remove residual moisture, and reweighed to calculate cooking loss. Cooking loss was calculated as follows:

$$Cooking\ loss = (initial\ weight - final\ weight)/initial weight$$

The WHC was tested as follows: approximately 20 g samples were weighed before being covered with gauze and filter papers, placed in Tenove Meat-1 (Beijing, China) and squeezed for 5 min, and then reweighed. Each sample was replicated in triplicate. The difference between the initial and final weights was used to calculate the WHC:

$$WHC = (initial\ weight - final\ weight)/initial weight$$

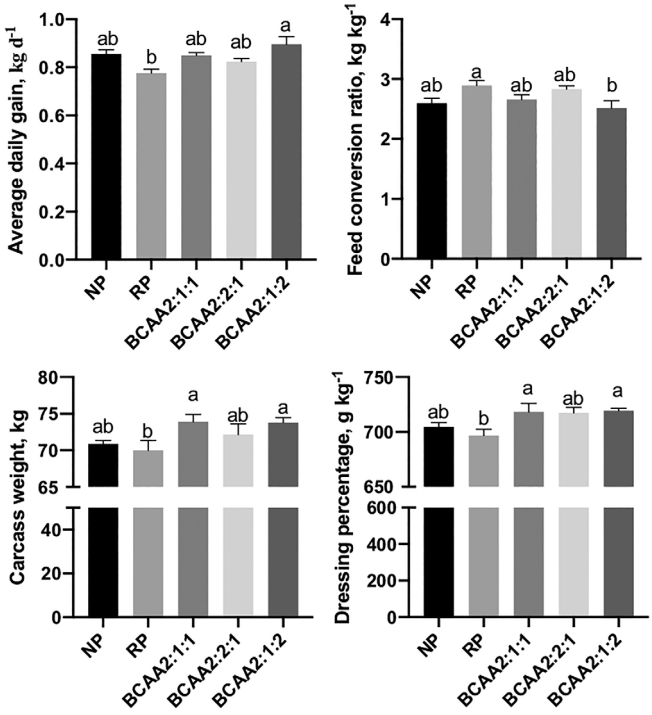

Figure 1. Analysis of average daily gain, feed conversion ratio, carcass weight, and dressing percentage of finishing pigs fed RP diets supplemented with branched-chain amino acids. NP, 160 g kg^{-1} crude protein level diet (NRC, 2012)[13]; RP, 120 g kg^{-1} crude protein level diet; BCAA2:1:1, RP diet supplemented with BCAAs to the ratio (Leu:Ile:Val) 2:1:1; BCAA2:2:1, RP diet supplemented with BCAAs to the ratio 2:2:1; BCAA2:1:2, RP diet supplemented with BCAAs to the ratio 2:1:2. Values with different letters (a–c) indicate significant differences among different treatments ($P < 0.05$). Values are means ± standard error of the mean (SEM) (n = 8).

IMF content analysis

Approximately 50 g of the meat sample from the LDM was sheared into small tubes, freeze dried, and ground into powder. The IMF content was analyzed by Soxhlet petroleum-ether extraction (Budwi Extraction System B-11, Budwi, Lausanne, Switzerland). The IMF content of freeze-dried meat was then converted to the IMF of fresh meat.

Muscle biochemical index assay

The levels of triglyceride (TG), lactate dehydrogenase (LDH), and myoglobin (MB) in the LDM were assayed using assay kits (Jiancheng, Nanjing, China). The total protein concentration was determined using a bicinchoninic acid (BCA) assay (Jiancheng, Nanjing, China). All experiments were performed according to the

Table 3. Meat quality of finishing pigs fed RP diets supplemented with branched-chain amino acids

Item[a]	NP[b]	RP	RP			SEM	*P*
			BCAA2:1:1	BCAA2:2:1	BCAA2:1:2		
pH 45 min	6.13	6.13	6.23	6.36	6.26	0.04	0.43
pH 24 h	5.54	5.58	5.55	5.54	5.56	0.01	0.84
L*- lightness	47.99	46.87	47.88	48.23	47.01	0.30	0.51
a*- redness	14.64	15.31	14.55	14.76	15.11	0.12	0.20
b*- yellowness	5.29	5.18	4.91	5.41	4.81	0.12	0.52
Shear force, N	53.65^{a}	43.79^{b}	47.45^{ab}	45.95^{ab}	38.54^{b}	1.50	0.04
WHC[c], g kg^{-1}	784.22^{b}	814.26^{a}	807.52^{ab}	782.23^{b}	818.31^{a}	4.72	0.03
Cooking loss, g kg^{-1}	155.05	152.38	153.91	156.44	154.01	1.15	0.86

[a] Different letters (a, b) within a row indicate the significant difference among different treatments ($P < 0.05$), n = 8.
[b] Diet treatment: NP, 160 g kg^{-1} crude protein level diet (NRC, 2012)[13]; RP, 120 g kg^{-1} crude protein level diet; BCAA2:1:1, RP diet supplemented with BCAAs to the ratio (Leu:Ile:Val) 2:1:1; BCAA2:2:1, RP diet supplemented with BCAAs to the ratio 2:2:1; BCAA2:1:2, RP diet supplemented with BCAAs to the ratio 2:1:2.
[c] WHC, water holding capacity.

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manufacturer's instructions. The values obtained were normalized to the total cellular protein content and expressed as micromoles per gram of protein.

Muscle fatty acid composition

The fatty acid profile of skeletal muscle was analyzed as follows: 150 mg of lyophilized muscle sample was extracted using 4 mL of chloracetyl methanol (chloracetyl : methanol = 1 : 10), and treated with 1 mL of *n*-hexane and 1 mL of internal standard FA solution (1 mg mL^{-1}, C11:0). After vortexing for 1 min, samples were kept in a water bath at 75 °C for 2 h, and then 5 mL of potassium carbonate solution (70 g L^{-1}) was added. The mixtures were centrifuged at 900×*g* for 5 min. The supernatant was analyzed by gas chromatography (HP 6890 series, Hewlett Packard, Avondale, PA, USA) using a DB-23 capillary column (122–2362; length, 60 m; internal diameter, 0.25 mm; film thickness, 0.25 μm; Agilent Technologies Inc., Santa Clara, CA, USA) and a flame ionization detector.

Calculation of health lipid indices

The total proportion of SFA was calculated as the sum of the percentages of myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids. The total proportion of MUFAs was calculated by summing the percentages of palmitoleic acid (C16:1), oleic acid (C18:1*n*9c), elaidic acid (C18:1*n*9t), and eicosenoic acid (C20:1). The total percentage of PUFAs included linoleic acid (C18:2*n*-6), γ-linolenic acid (C18:3*n*6), arachidonic acid (ARA, C20:4*n*6), α-linolenic acid (ALA, C18:3*n*3), eicosapentaenoic acid (EPA, C20:5*n*3), and docosahexaenoic acid (DHA, C22:6*n*3). The sum of all *n*-6 PUFAs, comprising linoleic acid (C18:2*n*6), γ-linolenic acid (C18:3*n*6), and ARA (C20:4*n*6) in our study, was divided by the sum of all *n*-3 PUFAs (ALA, EPA, and DHA) to calculate the *n*-6: *n*-3 PUFA ratio. The PUFA:SFA ratio was calculated by dividing the total proportion of PUFA by the total proportion of SFA. Lipid quality indices were calculated according to the following indices: atherogenicity (AI), thrombogenicity (TI), and peroxidisability (PI), desirable hypocholesterolemic fatty acids (DHFA), hypercholesterolemic saturated fatty acids (HSFA), and hypocholesterolemic and hypercholesterolemic fatty acids ratio (H_{po}/H_{per}), which were calculated as follows:[16]

$$AI=(4*C14:0+C16:0)/[\sum MUFA+\sum(n-6)+\sum(n-3)]$$

$$TI=(C14:0+C16:0+C18:0)/[0.5*\sum MUFA+0.5*\sum(n-6)+3*\sum(n-3)+\sum(n-3)/\sum(n-6)]$$

$$PI=(MUFA\times0.025)+(C18:2n6\times1)+[(C18:3n6+C18:3n3)\times2]+(C20:4n6\times4)+(C20:5n3\times6)+(C22:6n3\times8)$$

$$DHFA=MUFA+PUFA+C18:0$$

$$HSFA=C14:0+C16:0$$

$$Hpo/Hper=(C18:1+C18:2n6+C18:3n3)/(C14:0+C16:0)$$

Quantification of gene expression

Total RNA was extracted from the LDM using TRIzol reagent (R4115–02; Magen, Guangzhou, China) according to the manufacturer's instructions. The concentration and quality of RNA were measured using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). Then, RNA was reverse transcribed into cDNA using the First Strand cDNA Synthesis Kit (CW0741A; CW Biotech Inc., Beijing, China). The relative expression of the genes was quantified by real-time quantitative polymerase chain reaction (qPCR) (qTOWER 2.2 Real-Time PCR; Analytik Jena AG, Germany) with a Takara real-time polymerase chain reaction (PCR) Kit (RR096A; Takara Bio Inc., Tokyo, Japan). All the samples were measured in triplicate. The primer sequences for genes are presented in Table 2. The GAPDH gene was used to normalize the expression of target genes according to the formula $2^{-\Delta\Delta Ct}$, which was calculated as follows:

$$\Delta\Delta Ct=(CtTarget-CtGAPDH)treatment-(CtTarget-CtGAPDH)control$$

Immunoblotting analysis

The total protein extracted from the muscle tissue was used to measure the relative protein levels of ACC (21923-1-AP, Proteintech, USA), p-ACC (3661 s, Cell Signaling Technology, MA, USA), PPARγ (2435 s, Cell Signaling Technology), AMPKα (66536-1-Ig, Proteintech, USA), p-AMPKα (2535 s, Cell Signaling Technology), SIRT1(2310 s, Cell Signaling Technology), p-SIRT1 (2314s, Cell Signaling Technology), and PGC-α (ab54481, Abcam, UK) by the western blotting technique as previously described.[17] The secondary antibody was HRP goat anti-rabbit IgG (SA00001-2, Proteintech, USA). Protein bands were visualized using a chemiluminescent reagent (Pierce, Rockford, IL, USA) with a

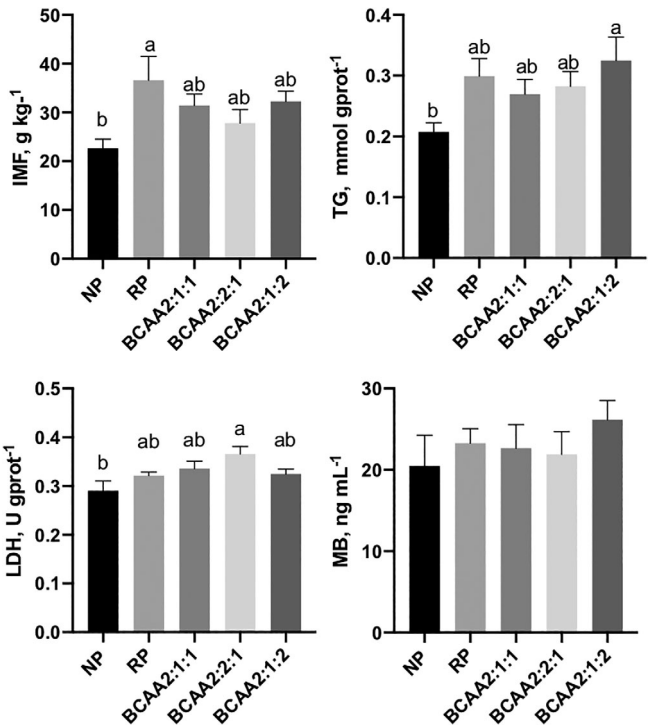

Figure 2. Analysis of intramuscular fat, triglyceride, lactate dehydrogenase, and myoglobin contents in LDM of finishing pigs fed RP diets supplemented with branched-chain amino acids. NP, 160 g kg^{-1} crude protein level diet (NRC, 2012)[13]; RP, 120 g kg^{-1} crude protein level diet; BCAA2:1:1, RP diet supplemented with BCAAs to the ratio (Leu:Ile:Val) 2:1:1; BCAA2:2:1, RP diet supplemented with BCAAs to the ratio 2:2:1; BCAA2:1:2, RP diet supplemented with BCAAs to the ratio 2:1:2. Values with different letters (a–c) indicate significant differences among different treatments ($P < 0.05$). Values are the means ± SEM (n = 8).

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Table 4. Free fatty acid compositions of *LDM* of finishing pigs fed RP diets supplemented with branched-chain amino acids

Item[a] (k kg^{-1})	NP[b]	RP	RP			SEM	*P*
			BCAA2:1:1	BCAA2:2:1	BCAA2:1:2		
Saturated fatty acid							
Myristic acid (C14:0)	11.44^{b}	13.20^{ab}	13.54^{a}	13.38^{ab}	11.80^{b}	0.30	0.03
Palmitic acid (C16:0)	235.98^{b}	257.66^{ab}	263.01^{a}	253.32^{ab}	236.33^{b}	3.47	0.02
Margaric acid (C17:0)	4.09	3.47	3.80	3.53	4.13	0.10	0.05
Stearic acid (C18:0)	130.53	129.12	132.61	134.78	130.91	1.57	0.88
Arachidic acid (C20:0)	1.64	1.99	1.86	1.88	1.83	0.06	0.41
∑SFA[c]	382.03	398.08	387.78	400.84	385.29	2.58	0.08
Unsaturated fatty acid							
Palmitoleic acid (C16:1)	32.38	33.16	36.42	32.67	32.29	0.79	0.42
Oleic acid (C18:1*n*9c)	370.39^{ab}	413.02^{a}	417.86^{a}	401.12^{ab}	364.91^{b}	7.27	0.03
Elaidic acid (C18:1*n*9t)	1.36^{ab}	1.67^{a}	1.71^{a}	1.51^{ab}	1.29^{b}	0.05	<0.01
Eicosenoic acid (C20:1)	6.63	7.11	7.91	7.47	6.65	0.20	0.24
∑MUFA	411.14^{b}	450.40^{ab}	463.90^{a}	442.72^{ab}	405.14^{b}	7.76	0.03
Linoleic acid (C18:2*n*6)	130.72	132.10	128.04	131.64	135.80	2.60	0.83
γ-linolenic acid (C18:3*n*6)	1.00	0.77	0.84	0.75	0.80	0.32	0.18
ARA (C20:4*n*6)	35.93^{a}	24.96^{b}	26.24^{ab}	24.34^{b}	24.15^{b}	1.47	0.01
∑*n*-6 PUFA	167.23	158.98	155.10	156.76	173.03	3.92	0.33
ALA (C18:3*n*3)	5.64	6.01	6.17	6.20	6.61	0.19	0.72
EPA (C20:5*n*3)	1.88^{ab}	1.20^{b}	1.31^{b}	1.37^{b}	2.28^{a}	0.12	<0.01
DHA (C22:6*n*3)	1.93^{a}	1.40^{ab}	1.30^{b}	1.15^{b}	1.50^{ab}	0.08	0.02
∑*n*-3 PUFA	9.54^{ab}	8.60^{b}	8.83^{b}	8.68^{b}	10.13^{a}	0.21	0.03
∑PUFA	187.91^{ab}	160.09^{b}	163.91^{b}	158.12^{b}	195.90^{a}	5.09	0.01

[a] Different letters (a, b, c) within a row indicate the significant difference among different treatments ($P < 0.05$), n = 8.
[b] Diet treatment: NP, 160 g kg^{-1} crude protein level diet (NRC, 2012)[13]; RP, 120 g kg^{-1} crude protein level diet; BCAA2:1:1, RP diet supplemented with BCAAs to the ratio (Leu:Ile:Val) 2:1:1; BCAA2:2:1, RP diet supplemented with BCAAs to the ratio 2:2:1; BCAA2:1:2, RP diet supplemented with BCAAs to the ratio 2:1:2.
[c] SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; ARA, arachidonic acid; ALA, *α*-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Table 5. Health lipid indices of *LDM* of finishing pigs fed RP diets supplemented with branched-chain amino acids

Item[a]	NP[b]	RP	RP			SEM	*P*
			BCAA2:1:1	BCAA2:2:1	BCAA2:1:2		
n-3/*n*-6 PUFA[c]	17.24	17.43	17.70	17.38	17.15	0.34	0.97
PUFA/SFA	0.54^{a}	0.43^{b}	0.46^{ab}	0.42^{b}	0.52^{a}	0.01	0.03
AI	0.47^{b}	0.51^{ab}	0.49^{ab}	0.52^{a}	0.47^{b}	0.01	<0.01
TI	0.74	0.73	0.69	0.77	0.75	0.01	0.47
PI	74.74	70.72	72.80	70.41	72.36	0.71	0.39
DHFA, g kg^{-1}	727.98^{ab}	706.80^{bc}	701.23^{c}	713.27^{abc}	731.95^{a}	3.71	0.02
HSFA, g kg^{-1}	247.41^{b}	270.86^{a}	276.56^{a}	266.70^{ab}	248.13^{b}	3.75	0.02
Hpo/Hper	2.11	1.99	2.02	1.97	2.11	0.02	0.10

[a] Different letters (a, b, c) within a row indicate the significant difference among different treatments ($P < 0.05$), n = 8.
[b] Diet treatment: NP, 160 g kg^{-1} crude protein level diet (NRC, 2012)[13]; RP, 120 g kg^{-1} crude protein level diet; BCAA2:1:1, RP diet supplemented with BCAAs to the ratio (Leu:Ile:Val) 2:1:1; BCAA2:2:1, RP diet supplemented with BCAAs to the ratio 2:2:1; BCAA2:1:2, RP diet supplemented with BCAAs to the ratio 2:1:2.
[c] SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; AI, atherogenicity; TI, thrombogenicity; PI, peroxidisability; DHFA, desirable hypocholesterolemic fatty acids; HSFA, hypercholesterolemic saturated fatty acids; H_{po}/H_{per}, hypocholesterolemic and hypercholesterolemic fatty acids ratio.

ChemiDoc XRS system (Bio-Rad, Philadelphia, PA, USA). The resultant signals were quantified using Alpha Imager 2200 software (Alpha Innotech Corp., San Leandro, CA, USA), and the data were normalized to the inner control, tubulin (2146s, Cell Signaling Technology).

Statistical analysis

All data obtained from the present study were analyzed by one-way analysis of variance (ANOVA) using IBM SPSS 26 software, as described earlier,[18] followed by Tukey's multiple-range test to determine treatment effects. The results are expressed as a

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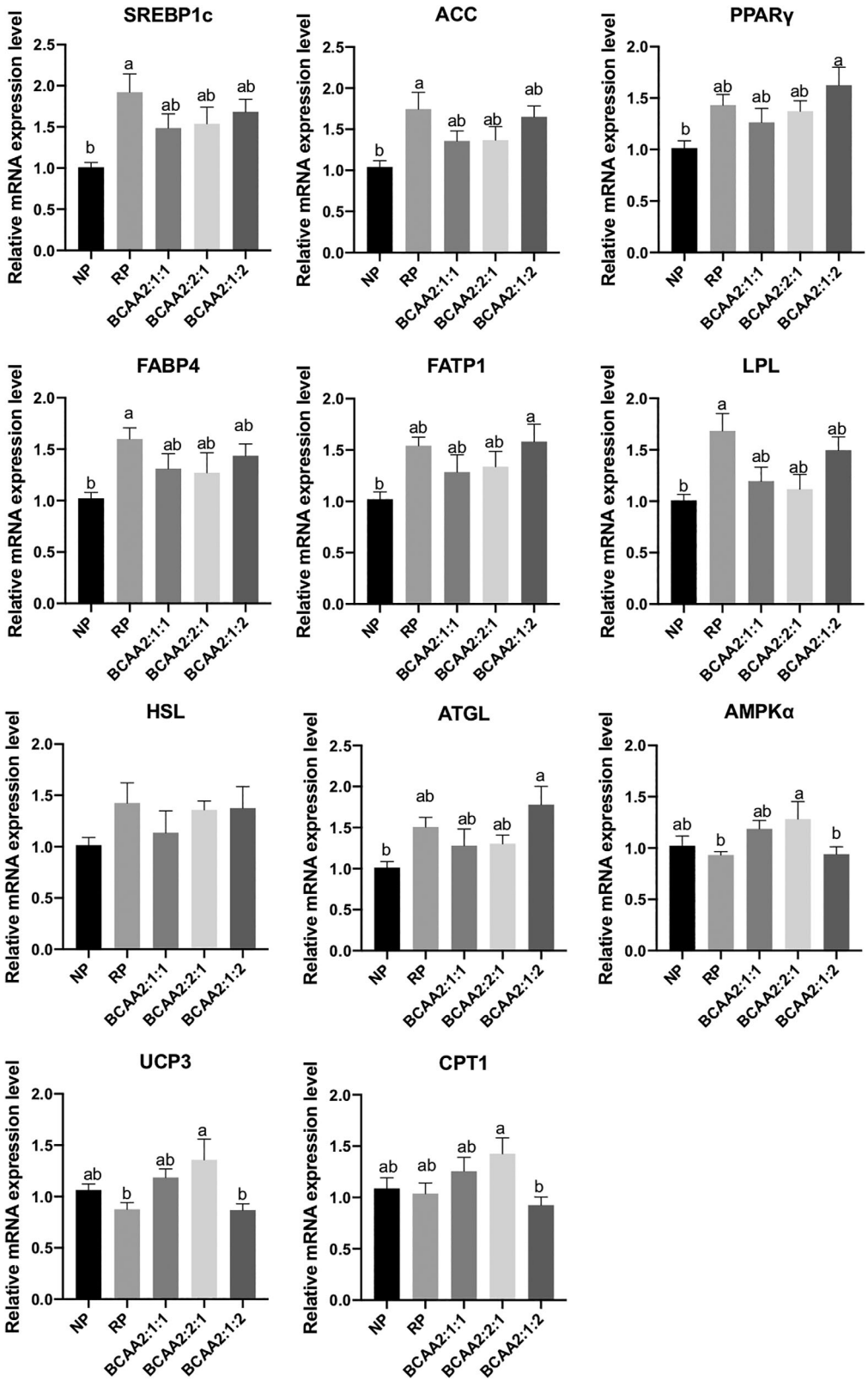

Figure 3. Relative mRNA levels of *SREBP1c*, *ACC*, *PPARγ*, *FABP4*, *FATP1*, *LPL*, *HSL*, *ATGL*, *AMPKα*, *UCP3*, and *CPT1* in LDM of finishing pigs fed RP diets supplemented with branched-chain amino acids. NP, 160 g kg^{-1} crude protein level diet (NRC, 2012)[13]; RP, 120 g kg^{-1} crude protein level diet; BCAA2:1:1, RP diet supplemented with BCAAs to the ratio (Leu:Ile:Val) 2:1:1; BCAA2:2:1, RP diet supplemented with BCAAs to the ratio 2:2:1; BCAA2:1:2, RP diet supplemented with BCAAs to the ratio 2:1:2. Values with different letters (a–c) indicate significant differences among different treatments ($P < 0.05$). Values are means ± SEM (n = 8).

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mean ± SEM and were regarded as statistically significant at $P < 0.05$.

RESULTS

Growth performance and carcass traits

As presented in Fig. 1, compared with the RP group, pigs in the restricted-protein diet supplemented with the BCAA ratio of 2:1:2 had significantly higher average daily gain ($P < 0.05$), carcass weight ($P < 0.05$), and dressing percentage ($P < 0.05$), and significant lower feed conversion ratio ($P < 0.05$), which performed best among the five treatments, even exceeding the NP group.

Meat quality traits

As indicated in Table 3, the meat quality traits were compared among the five treatment groups. In comparison to the NP group, the shear force in the RP group and the group with a restricted-protein diet supplemented with BCAA to a ratio of 2:1:2 was significantly decreased ($P < 0.05$), whereas the WHC in these two groups was significantly increased ($P < 0.05$). No significant differences were observed in other meat quality traits among the five groups.

IMF content

As the two meat quality traits of WHC and shear force represent meat tenderness and juiciness, we further tested the IMF content of the LDM, as shown in Fig. 2. Compared to the NP group, the RP group exhibited a significantly greater IMF content ($P < 0.05$). Besides, the three BCAA ratios in restricted-protein diets showed the same trend in improving IMF content.

Muscle metabolites

Triglyceride, LDH, and MB are essential metabolites for the determination of meat quality. As shown in Fig. 2, the TG content was evaluated in the LDM, and the results showed the same trend as the IMF content in the LDM. In comparison with the NP group, the group with a restricted-protein diet supplemented with BCAAs to the ratio of 2:1:2 exhibited a significantly higher TG content ($P < 0.05$). We also analyzed the activity of LDH, which is used as a marker of glycolytic and oxidative capacity in the LDM. Moreover, the group with a RP diet supplemented with BCAAs to 2:2:1 showed significantly increased LDH activity compared to the NP group ($P < 0.05$). Myoglobin is a versatile peroxidase that plays an important role in antioxidant defense in the LDM,[19] and is representative of meat color.[20] However, no significant difference was observed in the MB activity among the five groups.

Fatty acid composition of IMF in the LDM

Table 4 shows the fatty acid composition of the IMF. For SFA, compared with the NP group, the group with a restricted-protein diet supplemented with BCAAs to a ratio of 2:1:1 showed significantly increased C14:0 and C16:0 ($P < 0.05$) in IMF. For MUFA, compared with the NP group, the RP group and the group with a restricted-protein diet supplemented with BCAAs to a ratio of 2:1:1 showed significantly increased C18:1n9c and C18:1n9t ($P < 0.05$) in IMF. Compared with the NP group, the RP diet supplemented with BCAAs to a ratio of 2:1:1 showed significantly increased total MUFA ($P < 0.05$). For PUFA, compared with the RP group, the RP diet supplemented with BCAAs to the ratio of 2:1:2 showed significantly increased EPA ($P < 0.01$) in IMF. Compared with the NP group, the RP group and all three BCAA groups except the one with the ratio of 2:1:1 showed significantly decreased ARA ($P = 0.01$). Compared to the RP group, the RP diet supplemented with BCAAs to a ratio of 2:1:2 showed significantly increased total n-3 PUFA ($P < 0.05$), total PUFA ($P = 0.01$) and PUFA:SFA ratio ($P < 0.05$).

The health indices of the fatty acids are shown in Table 5. Among the health indices, AI and TI represent the effects of fatty acids on the incidence of atherosclerosis or thrombosis, respectively. Compared to the RP group, the NP group and the group fed a RP diet supplemented with BCAAs to a ratio of 2:1:2 showed significantly decreased AI ($P < 0.01$). Desirable hypocholesterolemic fatty acids (DHFA), also known as DFA, represent dietary fatty acids with a desirable neutral hypocholesterolemic effect in humans, whereas HSFA represents dietary fatty acids with an undesirable hypercholesterolemic effect in humans.[21] Compared with the RP group, the NP group and the group fed a RP diet supplemented with BCAAs to a ratio of 2:1:2 showed significantly increased DHFA ($P < 0.05$) and decreased HSFA ($P < 0.05$).

Relative mRNA expression level of lipid metabolism-related genes

As Fig. 3 illustrates, the RP group showed higher ($P < 0.05$) mRNA levels of *sterol regulatory element-binding protein 1 (SREPB1c)*, *acetyl-CoA carboxylase* (*ACC*), *fatty acid binding protein 4* (*FABP4*), and

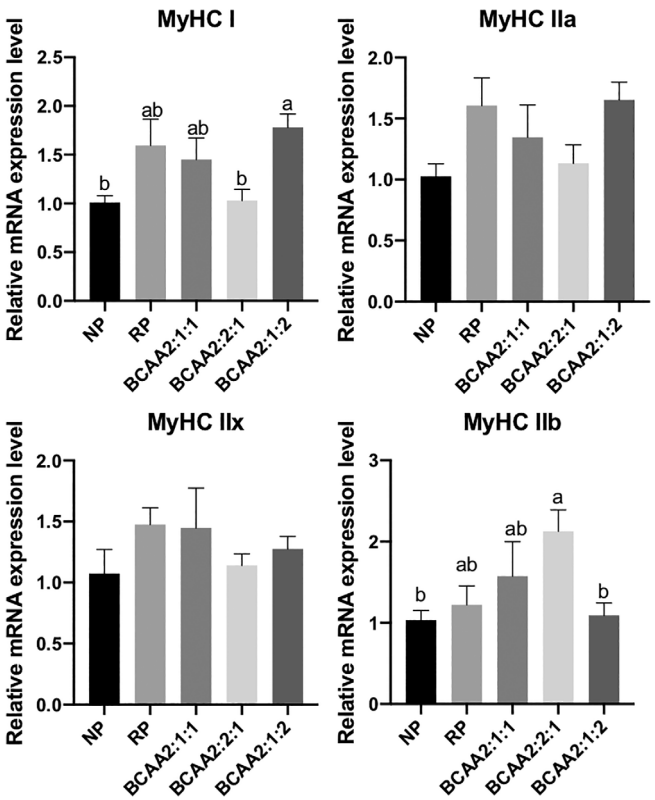

Figure 4. Relative mRNA levels of *MyHC* I, *MyHC IIa*, *MyHC IIx*, and *MyHC IIb* in LDM of finishing pigs fed RP diets supplemented with branched-chain amino acids. NP, 160 g kg^{-1} crude protein level diet (NRC, 2012)[13]; RP, 120 g kg^{-1} crude protein level diet; BCAA2:1:1, RP diet supplemented with BCAAs to the ratio (Leu:Ile:Val) 2:1:1; BCAA2:2:1, RP diet supplemented with BCAAs to the ratio 2:2:1; BCAA2:1:2, RP diet supplemented with BCAAs to the ratio 2:1:2. Values with different letters (a–c) indicate significant differences among different treatments ($P < 0.05$). Values are the means ± SEM (n = 8).

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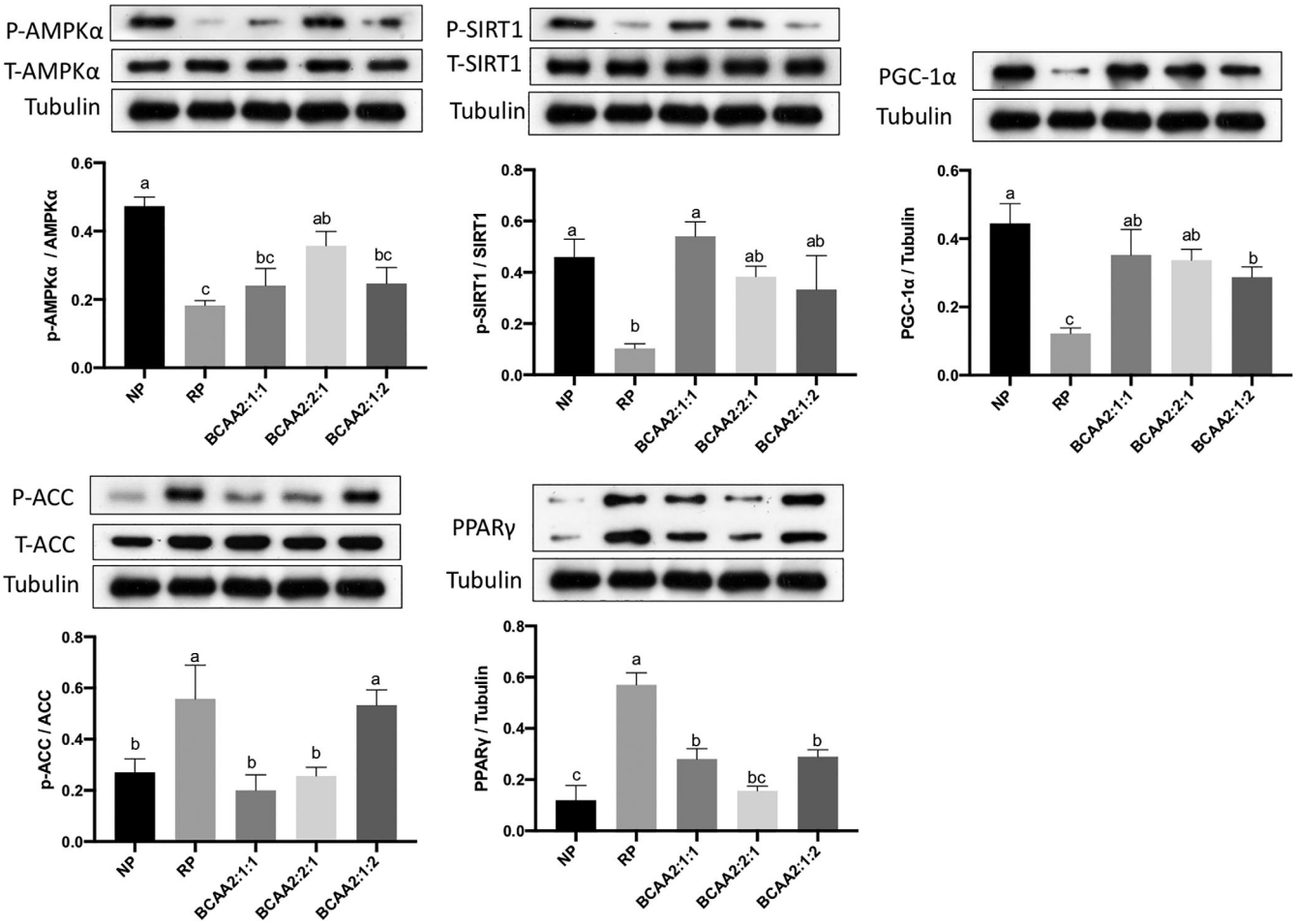

Figure 5. Relative protein levels of AMPKα, SIRT1, PGC-1α, ACC, and PPARγ in LDM of finishing pigs fed RP diets supplemented with branched-chain amino acids. NP, 160 g kg^{-1} crude protein level diet (NRC, 2012)[13]; RP, 120 g kg^{-1} crude protein level diet; BCAA2:1:1, RP diet supplemented with BCAAs to the ratio (Leu:Ile:Val) 2:1:1; BCAA2:2:1, RP diet supplemented with BCAAs to the ratio 2:2:1; BCAA2:1:2, RP diet supplemented with BCAAs to the ratio 2:1:2. Values with different letters (a–c) indicate significant differences among different treatments ($P < 0.05$). Values are the means ± SEM (n = 8).

lipoprotein lipase (LPL) in the LDM than in the NP group. The RP diet supplemented with BCAAs to a ratio of 2:1:2 showed higher ($P < 0.05$) mRNA levels of *peroxisome proliferation-activated receptor-γ* (*PPARγ*), *fatty acid transport protein 1* (*FATP1*), and *adipose tissue triglyceride lipase* (*ATGL*) in the LDM than in the NP group. In terms of energy metabolism, the mRNA levels of *AMPKα*, *uncoupling protein type 3 (UCP3)*, and *carnitine palmitoyl transferase 1* (*CPT1*) in the group fed a RP diet supplemented with BCAAs to a ratio of 2:2:1 were the highest among the five treatments.

Relative mRNA expression level of myosin heavy-chain isoform

We further analyzed the gene expression of the myosin heavy-chain isoform to determine whether increased IMF was accompanied by muscle fiber type conversion. As presented in Fig. 4, compared to the NP group, the mRNA expression of *MyHC I* was found to increase significantly in the RP diet supplemented with BCAAs to a ratio of 2:1:2 ($P < 0.05$), whereas the mRNA expression of *MyHC IIb* was found to increase significantly in the group fed a RP diet supplemented with BCAAs to a ratio of 2:2:1 ($P < 0.05$). No significant changes in the gene expression of *MyHC IIa* and *MyHC IIx* were observed among the five treatment groups.

Expression of proteins involved in energy and lipid metabolism

As expected, in comparison with the NP group, the protein level of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in the LDM was found to decrease significantly ($P < 0.05$) in the RP diet supplemented with BCAAs to a ratio of 2:1:2, whereas the protein level of P-SIRT1 showed a similar trend.

DISCUSSION

Future trends in the pork market largely depend on consumer demand for healthier, safer, and better quality meat.[1] Specifically, tenderness and juiciness are the most common indicators used by consumers to judge meat quality, which is commonly determined by IMF.[10] In this study, as the dietary protein level of finishing pigs decreased from 160 to 120 g kg^{-1}, the RP diet remarkably increased WHC and decreased shear force, simultaneously significantly increasing IMF content. At the same time, the RP diets supplemented with BCAAs showed a similar trend, especially for the ratio of 2:1:2. In the RP and BCAA 2:1:2 treatments, the IMF content and muscle TG abundance were also significantly improved, demonstrating that the addition of balanced BCAAs to the RP diet to a

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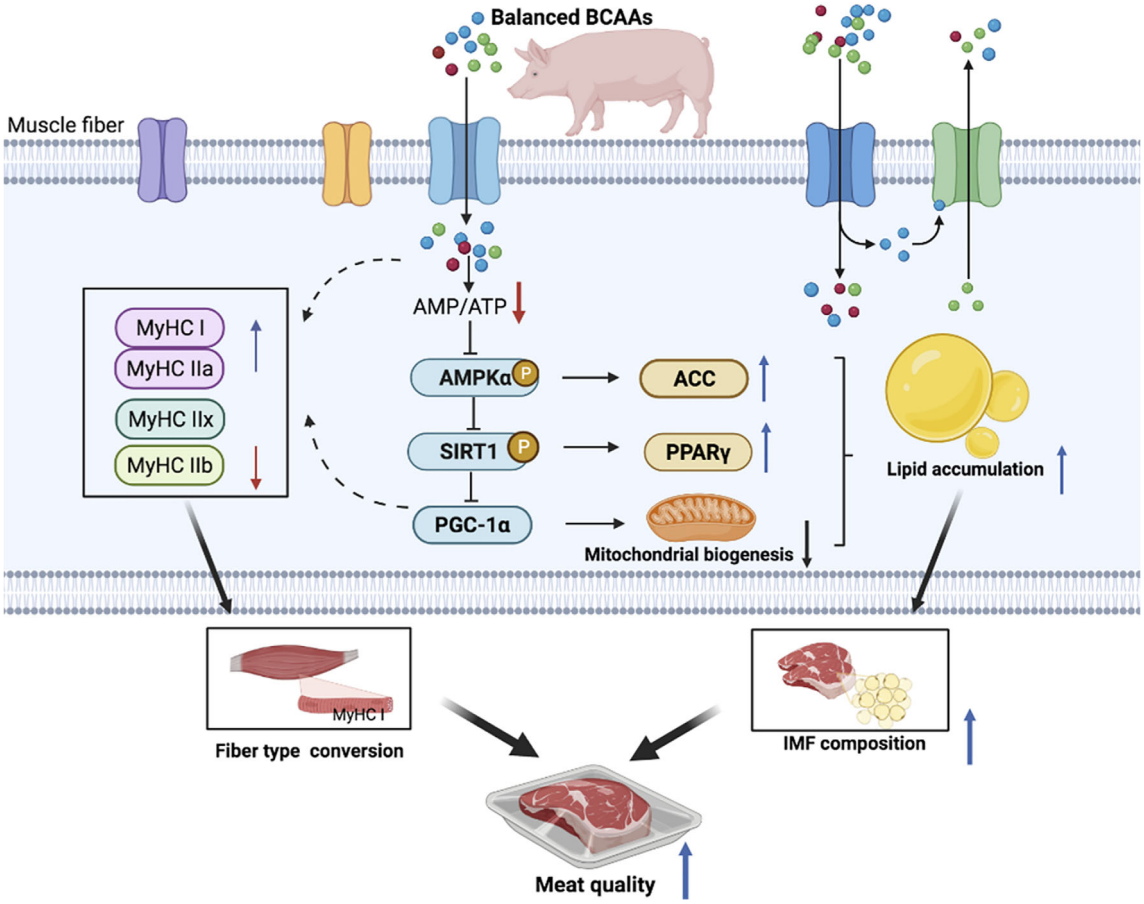

Figure 6. Graphical representation of the anticipated mechanism of the effects of protein-restricted diets supplemented with balanced BCAA ratios on meat quality of finishing pig.

ratio of 2:1:2 could increase IMF in muscles, thereby contributing to the juicy, tender, and flavorful characteristics of pork. We also found that pigs with the RP diet supplemented with BCAAs had an increasing growth and carcass performance, indicating that supplementing with balanced BCAAs not only ameliorated the poor performance caused by the RP diet but also maintained excellent meat quality.

Fatty acids including SFAs, MUFAs, and PUFAs, are major sources of energy in skeletal muscle and key factors affecting IMF composition.[22] Meanwhile, fatty acid composition is closely associated with the occurrence and development of metabolic diseases, such as cardiovascular disease, obesity, and diabetes.[23] Nowadays, dietary guidelines for humans recommend limiting the SFA-rich foods, and increasing the intake of unsaturated fatty acids (UFA).[24] Surprisingly, by adding BCAAs to the RP diet to a ratio of 2:1:1, the total MUFA content was significantly increased. Similarly, by adding BCAAs to the RP diet to a ratio of 2:1:2, the total PUFA and PUFA-to-SFA ratio were significantly increased. Therefore, RP diets supplemented with BCAAs of 2:1:1 or 2:1:2 may have beneficial effects on lipid profiles and cardiovascular disease risk factors, allowing consumers to take precautions against obesity, hypercholesterolemia, and cancer. In view of its cardioprotective benefits, particular attention has been paid to *n*-3 PUFA, specifically EPA and DHA.[25] In the RP diets, the EPA and DHA composition were both decreased. Surprisingly, the addition of BCAAs to an RP diet to a ratio of 2:1:2 increased the content of these two important healthful indicators. It is also worth noting that RP diets could exacerbate some health indicators, such as increased AI and HSFA, which represent atherogenicity and may have an undesirable hypercholesterolemic effect in humans, as well as decreased DHFA, which has a desirable neutral hypocholesterolemic effect in humans.[16] Surprisingly, the addition of BCAAs to an RP diet to a ratio of 2:1:2 could reverse these three health indicators. Hence, RP diets supplemented with balanced BCAAs, especially the ratio of 2:1:2 may be more in line with the demand of consumers for healthy meat containing lower SFA, as well as higher MUFA and *n*-3 PUFA.

The concentration of fatty acids in the muscle is mainly regulated by key genes encoding specific metabolic enzymes related to lipid metabolism, such as *SREBP-1c*, *ACC*, *PPARγ*, and *LPL*, controlling TG partitioning between adipose tissue and muscles, thus affecting IMF deposition in muscles. *SREBP-1c* promotes fatty acid synthesis by inducing the expression of *ACC* and may promote the transcription of *PPARγ*, thereby regulating fatty acid biosynthesis. Activated *PPARγ* promotes the expression of its target gene *LPL*, a rate-limiting enzyme, whose mutation can significantly affect fatty acid composition in muscles.[26–29] Consistent with the IMF content, the observation that the expression of *SREBP-1c*, *ACC*, *PPARγ*, and *LPL* increased in pigs fed a RP diet with balanced BCAAs, indicating increased lipogenesis and the uptake of fatty acids in skeletal muscle in pigs fed the RP diets containing balanced BCAAs, particularly the ratio of 2:1:2. *CPT1* is the rate-

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limiting enzyme regulating β-oxidation in the mitochondria,[30] which is decreased in the RP diet supplemented with BCAAs to a ratio of 2:1:2. Moreover, *FABP4* and *FATP1* are mainly involved in fatty acid transport,[31,32] which were increased in the supplementation of BCAAs to RP diets, particularly to the ratio of 2:1:2, indicating that these treatments showed more intense levels of lipid metabolism in the LDM. Unexpectedly, when BCAAs were added to RP diets, the expression of *ATGL*, a lipolysis-related gene,[33] also increased. This may be because IMF deposition depends on the balance of TG synthesis and degradation, involving not only lipogenesis but also fatty acid transport and lipolysis. In comparison with the other two BCAA ratios, RP diets supplemented with BCAAs to a ratio of 2:1:2 showed the strongest lipid metabolism level and performed the best in terms of IMF deposition. This phenomenon may be due to higher content of Val in such ratio and Val's intermediate catabolic product, 3-hydroxyisobutyrate (3-HIB), which was reported to promote trans-endothelial fatty acid transport,[34] further linking the fatty acid flux and the BCAA metabolism.

It is reported that AMPKα, SIRT1, and PGC-1α are associated with IMF accumulation.[18,35,36] Consistent with the mRNA expression level, we confirmed that in RP diets, especially those supplemented with BCAAs to a ratio of 2:1:2, the protein levels of phospho-AMPKα (p-AMPKα)/AMPKα and p-ACC/ACC were significantly decreased and increased, respectively. The activation of AMPKα promotes lipogenesis while limiting lipid oxidation through the phosphorylation and promotion of ACC, a key enzyme in lipogenesis, leading to IMF deposition.[26] Activated AMPKα can also phosphorylate SIRT1,[11] a major regulatory factor of energy metabolism, which was reported to inhibit the transcription of PPARγ, thereby inhibiting adipocyte differentiation and reducing adipogenesis.[18,27] Similarly, we found that, in RP diets, especially supplemented with BCAAs to the ratio of 2:1:2, p-SIRT1/SIRT1 were significantly decreased, whereas p-PPARγ/ PPARγ were significantly increased, thereby enhancing TG and IMF accumulation. Activated AMPKα and SIRT1 could both phosphorylate PGC-1α, the major energy-related factor of mitochondrial biogenesis and fatty acid β-oxidation in skeletal muscle.[36] Consistent with these expression patterns, the expression of PGC-1α and *UCP3* significantly decreased in RP diets supplemented with BCAAs to the ratio of 2:1:2, indicating that mitochondrial biogenesis was inhibited in these treatments. We therefore further confirmed that dietary balanced BCAAs in RP diets could promote IMF deposition through the AMPK-SIRT1-PGC-1α axis, thereby phosphorylating ACC and PPARγ, increasing fat accumulation, and reducing mitochondrial biogenesis in LDM, thus providing important mechanical insights into improving meat quality (Fig. 6).

As BCAA ratios significantly improved the IMF content and UFA composition, which are positively correlated with meat tenderness, we further investigated whether BCAAs influence meat tenderness by regulating muscle fiber conversion. In this study, we found that the expression of *MyHC I* increased whereas that of *MyHC IIb* decreased in RP diets, especially supplemented with BCAAs to the ratio of 2:1:2, which is consistent with the shear force trait, showing higher tenderness. Indeed, the cherry-red color of red meat is often considered as an indicator of health and taste, which is associated with higher amounts of oxidative muscle fibers.[37] The proportion of oxidized fibers in muscle is negatively correlated with LDH activity,[38] which is consistent with our results. Some studies have suggested that PGC-1a stimulates fiber-type conversion in muscles.[5,39–41] However, in our study, the expression levels of *MyHC I* and PGC-1α were inconsistent. This may be because an increase in PGC-1α expression is not the only factor that promotes muscle fiber conversion. Although a strong relationship has been reported between the fiber type composition and IMF deposition,[42,43] whether this relationship is modulated by the AMPK-SIRT1-PGC-1α axis or other BCAA metabolites needs further investigation.

As long as the supply of amino acids matches demand, the amino acid balance is achieved, and their utilization and metabolism are maximized.[44] Indeed, we previously found the potential balanced BCAA ratios (1: 0.75: 0.75- 1: 0.25: 0.25) could induce muscle more oxidative in growing pigs.[5] Based on that ratio range, as well as according to NRC (2012),[13] we designed the ratios in finishing pigs in this experiment (Leu : Ile : Val = 2 : 1 : 1, 2 : 2 : 1, 2 : 1 : 2). In fact, we would like to study that when the supplementation of Leu is sufficient, whether the increase or the other two BCAAs (Ile or Val) will affect the metabolism, thus deeply revealing the importance of the balance of BCAAs. In this study, our results showed that the BCAA ratio of 2:1:2 performed better, indicating that the increase of Val may be beneficial to the balance of BCAAs, thus enhancing meat quality.

CONCLUSIONS

In summary, our data highlight the importance of RP diets supplemented with balanced BCAA ratios, especially the Leu : Ile : Val ratio of 2 : 1 : 2, for improving meat tenderness and juiciness, thus modulating meat quality by increasing IMF deposition and fiber type conversion in finishing pigs, which may be associated with the AMPK-SIRT1-PGC-1α axis. Although the mechanism underlying pork quality is extremely complex, these results provide a cross-regulatory molecular basis of nutritional regulation, highlighting the effects of supplementation with RP diets-balanced BCAAs on meat quality, which is likely to be of great significance for elucidating the precise metabolic regulation network of meat quality.

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