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ORIGINAL ARTICLE

Co-dependence of genotype and dietary protein intake to affect expression on amino acid/peptide transporters in porcine skeletal muscle

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Abstract A total of 96 barrows (48 pure-bred Bama mini-pigs representing fatty genotype, and 48 Landrace pigs representing lean genotype) were randomly assigned to either a low- or adequate-protein treatment diet. The experimental period commenced at 5 weeks of age and extended to the finishing period. After euthanasia, blood and skeletal muscle samples were collected from pigs at the nursery, growing, and finishing phases. Our results indicate that the concentrations of free AAs in the plasma and muscle decreased as the age of the pigs increased. In addition, a strain × growth phase interaction ($P < 0.05$) was observed for the free AA pool in the plasma and muscle. The low-protein diet upregulated ($P < 0.05$) the mRNA levels for T1R1/T1R3 involved in glutamate binding, but downregulated ($P < 0.05$) the mRNA levels for PAT1, PAT2, and ASCT2, which transport neutral AAs into muscles. Bama mini-pigs had higher ($P < 0.05$) mRNA levels for LAT1, SNAT2, and EAAC1, but a lower ($P < 0.05$) mRNA level for PepT1, compared with Landrace pigs. Collectively, our findings indicate that adequate provision of dietary protein plays an important role in regulating profiles of free AA pools and expression of key AA/peptide transporters/transceptors in a genotype- and tissue-specific manner.

Keywords Bama mini-pig · Dietary protein · Skeletal muscle · AA transporter · AA receptor

Introduction

A balanced supply of dietary proteins and amino acids (AAs) is necessary for the optimal growth, development, and reproduction of animals (Deng et al. 2007a, b, 2009; Wu et al. 2010, 2011; Wu 2010, 2014), as well as enhancing feed efficiencies in livestock production and minimizing its impact on environmental health (Yin and Tan 2010; Wu et al. 2014a, b; Chen et al. 2014). Recent studies have demonstrated that AAs are not only building blocks for protein

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synthesis in cells and signaling molecules (Wu et al. 2010, 2012, 2013a, b, 2015; Li et al. 2011; Wang et al. 2014a, b, 2015a, b) but also regulators of gene expression (He et al. 2013; Chen et al. 2014; Zhang et al. 2013; Ren et al. 2013a, b, c, 2014a, b; Tan et al. 2011; Yang et al. 2013; Feng et al. 2014; Tang et al. 2014, Yin et al. 2014; Kong et al. 2014) and protein posttranslational regulation (Kong et al. 2012). Additionally, AAs are key precursors for the biosynthesis of hormones and low-molecular-weight nitrogenous substances (Wu 2013a, b). These physiological functions depend on the optimal concentrations of AAs and their metabolites, including peptides, polyamines, glutathione, taurine, thyroid hormones, and serotonin in the blood, cells, and tissues (Ren et al. 2013a, b; Wu et al. 2014c).

Through changes in intracellular concentrations, AAs act as signaling molecules that regulate metabolic pathways involved in muscle growth (Sancak et al. 2008) and other tissues (Brosnan and Brosnan 2013; Fernstrom 2013; San Gabriel and Uneyama 2013). This signaling function plays an important role in the regulation of skeletal muscle hypertrophy in monogastric animals through the activation of specific cell-signaling pathways (Tan et al. 2009; Yao et al. 2008). For example, the mammalian target of rapamycin (mTOR) represents a crucial kinase for protein synthesis and cell growth (Wang and Proud 2011; Zoncu et al. 2011). AA uptake requires numerous transport systems that vary in their substrate specificity, affinity, and velocity (Wu 2013a). Dietary proteins are digested in the small intestine, which releases free AAs and oligo-peptides that are absorbed by enterocytes and subsequently utilized by the liver and other organs (Davila et al. 2013; Jobgen et al. 2006). Thus, after a meal, marked changes are noted in the concentrations of free AAs in the intestinal lumen, blood plasma, and intracellular and extracellular spaces (Blachier et al. 2009).

As pig strains may vary in their capacity to recognize changes in AA concentration in body fluids, the utilization of free AAs may also differ among pig strains. The Bama mini-pig (*Sus scrofa domestica*) is an indigenous Chinese "fatty" strain, mainly found in Bama County of the Guangxi Province of China. Because their anatomical, physiological, and metabolic characteristics are similar to those of humans, Bama mini-pigs have been widely used in experiments for extrapolation to human metabolism and physiology (Kawaguchi et al. 2011; Liu et al. 2008). In contrast, the leaner Landrace pig has rapid growth rate and yields leaner meat, which are nutritional and commercial advantages. We hypothesized that differences between these two strains of pigs in their muscle growth, meat quality, and intermuscular adipose deposition (Liu et al. 2015) may lead to dietary protein-dependent differences in AA metabolism. The major objectives of this study were to measure free AA pools in the plasma and muscle tissues, as well as mRNA levels for AA and peptide transporters and receptors in the skeletal muscles of Bama mini-pigs and Landrace pigs fed low- or adequate-protein diets.

Materials and methods

Animals, diets, and treatments

All experiments were carried out in accordance with Chinese guidelines for animal welfare, and experimental protocols were approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, the Chinese Academy of Sciences (Yin et al. 2009). A total of 96 barrows [48 purebred Bama mini-pigs with an average initial body weight (BW) of 3.38 ± 0.96 kg, and 48 Landrace pigs with an average initial BW of 7.68 ± 0.89 kg] were fed the test diets from 5 weeks of age until they attained their finishing BW. The experiment was a 2 × 2 factorial arrangement, with two pig strains (Bama mini-pigs and Landrace pigs) and two diets [the National Research Council (NRC) diet and the Chinese conventional diet (GB)], resulting four dietary treatments (Table 1). Forty-eight piglets from each strain were randomly assigned to one of the two dietary treatments. The NRC diet, which had a higher protein level, was formulated to meet the nutrient requirements outlined by the NRC (2012), whereas the GB diet was formulated to conform to the recommendations of the Chinese National Feeding Standard for Swine (Ministry of Agriculture of the People's Republic of China, 2004), and had a lower protein content (Table 2). The dietary AA composition, which was determined as described by Dai et al. (2014), is shown in Table 3. All pigs had free access to drinking water and their experimental diets (Yin et al. 2015). The room temperature was maintained at 25–27 °C. All pigs were fed three times a day (0800, 1300, and 1800) (Li et al. 2015).

Sample collection

Body weights for nursery, growing, and finishing phases in Landrace pigs were in the ranges of 7–20, 20–50, and 50–90 kg, respectively, whereas for Bama mini-pigs, they were in the ranges of 3–15, 15–35, and 35–55 kg, respectively (Table 1). At the end of each phase, eight pigs from each treatment group were randomly sampled. Briefly, after recording pre-slaughter BW and fasting the animals for 12 h, blood samples were obtained from the jugular vein and placed in 10 mL centrifuge tubes containing sodium heparin (14.3 USP units/mL) (Xiao 2015). The samples were then centrifuged at 900×*g* for 10 min at 4 °C to recover plasma, which was stored at −20 °C until analysis of free AAs was performed. The pigs were then placed under general anesthesia and killed by jugular vein

Table 1 Dietary treatments for Landrace pigs and Bama mini-pigs

Items	Landrace pig			Bama mini-pig		
	BW (kg)	GB diet group	NRC diet group	BW (kg)	GB diet group	NRC diet group
Nursery phase	7–20	GB diet 1	NRC diet 1	3–15	GB diet 1	NRC diet 1
Growing phase	20–50	GB diet 2	NRC diet 2	15–35	GB diet 2	NRC diet 2
Finishing phase	50–90	GB diet 3	NRC diet 3	35–55	GB diet 3	NRC diet 3

GB diet, the Chinese conventional diet; NRC diet, recommended by National Research Council (2012); BW, body weight

Table 2 Ingredients and nutrient levels in experimental diets

Items	NRC diet 1	NRC diet 2	NRC diet 3	GB diet 1	GB diet 2	GB diet 3
Ingredients (%)						
Corn	62.80	66.00	69.50	63.00	60.00	66.00
Soybean meal, 42 % CP	26.00	28.00	23.00	25.00	26.50	21.00
Fish meal, 62 % CP	7.00	2.00	–	3.00	–	–
Wheat bran	–	–	3.00	6.34	10.75	10.50
Soybean oil	1.95	1.50	2.10	–	–	–
$CaHPO_4$	0.45	0.70	0.65	0.80	0.80	0.50
$CaCO_3$	0.50	0.50	0.45	0.56	0.65	0.70
Salt	0.30	0.30	0.30	0.30	0.30	0.30
Premix[a]	1.00	1.00	1.00	1.00	1.00	1.00
Nutrient levels						
Digestible energy (MJ/kg)	14.22	14.21	14.22	13.46	13.40	13.40
Crude protein[b] (%)	20.06	18.01	15.11	18.03	16.05	13.46
Total calcium (%)	0.75	0.62	0.50	0.69	0.62	0.56
Available phosphorus (%)	0.39	0.28	0.21	0.21	0.13	0.12

[a] Premix provided for 1 kg of the complete diet: Cu (as copper sulfate), 10 mg; Fe (as ferrous sulfate), 100 mg; Se (as sodium selenite), 0.30 mg; Zn (as zinc oxide), 100 mg; Mn (as manganese sulfate), 10 mg; vitamin D_3, 9.65 μg; vitamin A, 925.8 μg; vitamin E, 15.4 mg; vitamin K_3, 2.3 mg; vitamin B_2, 3.9 mg; D-calcium pantothenate, 15.4 mg; nicotinic acid, 23 mg; choline, 80 mg; vitamin B_{12}, 0.016 mg

[b] Analyzed values for crude protein, and calculated values for other nutrients

injection of 4 % sodium pentobarbital solution (40 mg/kg BW). After the head, legs, tail, and viscera were removed, the carcass was split longitudinally. Samples of the *longissimus dorsi* (LDM) and *biceps femoris* (BFM) muscles on the right side of the carcass were collected immediately and visible intermuscular adipose tissue was carefully removed (Li et al. 2007; Yang et al. 2005). The samples were snap-frozen in liquid nitrogen, and stored at −80 °C for subsequent analysis (Chen et al. 2011).

Determination of free amino acids in plasma

Plasma free AA concentrations were determined as previously described (Kong et al. 2009). Briefly, 1 mL of the plasma sample and 2.5 mL of 7.5 % trichloroacetic acid solution were mixed thoroughly and centrifuged at 12,000×*g* and 4 °C for 15 min (Ren et al. 2014c). The supernatant fluid was collected for analysis of free AAs by an ion-exchange AA analyzer (L8800, Hitachi, Tokyo, Japan).

Determination of free amino acids in muscle

To measure the concentrations of free AAs in muscle tissue, approximately 1 g of freeze-dried muscle was homogenized in 10 mL of 10 mmol/L hydrochloric acid. The solution was adjusted to a final volume of 25 mL by adding 10 mmol/L hydrochloric acid. After centrifuging at 12,000×*g* for 10 min, 2 mL of the supernatant liquid was mixed with 2 mL of 8 % 5-sulfosalicylic acid. After centrifuging at 12,000×*g* for 10 min, the supernatant fluid was filtered through a 0.45-μm membrane before analysis of AAs using an ion-exchange AA analyzer (L8800, Hitachi, Tokyo, Japan).

RNA extraction and cDNA synthesis

Total RNA was isolated from the LDM and BFM tissues frozen in liquid N using the TRIzol reagent (Invitrogen-Life Technologies, Carlsbad, CA, USA) and treated with DNase

Table 3 Amino acid composition in experimental diets for pigs (mg/g, as fed-basis)

Items	NRC diet 1	NRC diet 2	NRC diet 3	GB diet 1	GB diet 2	GB diet 3
Essential AA						
Arg	9.65	9.25	7.53	8.66	8.79	6.86
His	4.97	4.83	3.94	4.66	4.45	3.66
Ile	5.68	5.47	4.48	5.17	5.19	3.97
Leu	14.95	15.07	12.78	14.57	13.67	12.11
Lys	8.56	8.03	6.12	7.61	7.94	5.50
Met	2.49	1.77	2.13	2.32	1.74	1.34
Phe	6.90	7.47	5.78	6.88	6.96	5.50
Thr	6.06	5.58	4.39	5.44	5.28	4.06
Val	8.20	7.20	6.51	7.66	6.77	5.66
Total EAA	67.46	64.68	53.66	62.98	60.78	48.63
Non-essential AA						
Ala	11.26	9.19	8.80	10.59	8.74	7.84
Asp[a]	16.43	16.16	12.96	15.40	15.39	12.06
Cys	3.42	2.54	3.21	3.41	2.42	2.66
Glu[b]	37.06	38.02	31.06	37.06	35.94	29.94
Gly	7.66	6.77	5.30	6.89	6.36	4.91
Pro	18.76	18.18	14.94	17.91	16.99	14.00
Ser	5.90	6.43	4.62	5.72	5.86	4.57
Tyr	5.29	5.19	4.49	4.67	4.77	4.06
Total NEAA	105.78	102.50	85.38	101.66	96.48	80.03
Total AA	173.23	167.18	139.05	164.63	157.26	128.66

[a] Including aspartate and asparagine

[b] Including glutamate and glutamine

I (Invitrogen) according to the manufacturer's instructions. The RNA quality was confirmed with 1 % agarose gel electrophoresis and stained with 10 μg/mL ethidium bromide. The RNA had an OD260:OD280 ratio between 1.8 and 2.0 (Feng et al. 2015). The first-strand cDNA was synthesized with Oligo (dT) 20 and Superscript II reverse-transcriptase (Invitrogen), according to the manufacturer's instructions.

Determination of mRNA levels in muscle

Primers for the selected genes were designed using the Primer 5.0 software (Table 4). Real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed using the SYBR Green detection kit (TaKaRa, Japan), which contained $MgCl_2$, dNTP, and HotStar Taq Polymerase as in our previous study (Liu et al. 2015). Briefly, an aliquot (2 μL) of a cDNA template (corresponding to 25 ng of total RNA) solution was added to a total volume of 10 μL containing 5 μL SYBR Green mix, 0.2 μL ROX Reference Dye (50 X), and 0.2 μL of either forward or reverse primers. After a pre-denaturation program (10 s at 95 °C), 40 cycles of amplification were performed (95 °C for 10 s followed by 60 °C for 20 s), followed by a melting curve program (60–99 °C with a heating rate of 0.1 °C/s and fluorescence measurement). The fluorescence signal was detected by the ABI Prism 7900 HT (Applied Biosystems, Marsiling Industrial Estate Road 3, Singapore). A melting curve was generated for each sample at the end of each run to ensure the purity of the amplified products. The amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in each sample was used to normalize the mRNA levels of the selected genes. The relative expression ratio (R) of mRNA was calculated using the following formula:

$$R = 2^{-\Delta\Delta C_t\,(\text{sample}-\text{control})}$$

where $\Delta\Delta C_t$ (sample − control) = (C_t target gene − C_t GAPDH) for the sample − (C_t target gene − C_t GAPDH) for the control.

RT-PCR efficiencies were determined by the amplification of a series of dilutions of cDNA according to the equation $10^{(-1/\text{slope})}$, as described by Bustin et al. (2009), and were found to be consistent between target mRNA and GAPDH. For the negative controls, cDNA was replaced with water (Wang et al. 2009).

Statistical analysis

Data were analyzed by multifactor ANOVA using the GLM procedure of SAS 9.1 for Windows (SAS Institute Inc.,

Table 4 Primers used for real-time PCR in the study

Gene	Accession no.	Primers	Size (bp)
T1R1	XM_005656004	S: 5′-TCACTGGGCTTAAGGCTGG-3′	92
		A: 5′-TTCTCTGGCAGGTCCTTACCC-3′	
T1R3	NM_001113288	S: 5′-GTGGAGGAAATCAACAACGGAT-3′	156
		A: 5′-GTAGTCGCAGTAGGTGGCAATG-3′	
Calpain-3	NM_214171	S: 5′-ATGCCGACTGTCATTAGCG -3′	393
		A: 5′-CAGAAACCAGCAGTCCCCT-3′	
mTOR	XM_003127584	S: 5′-CGCGCCATCGCCACTGAGGAC-3′	90
		A: 5′-CAGCTGCCACTCTCCAAGTTTCA-3′	
PAT1	XM_003134140	S: 5′-TGTGGACTTCTTCCTAATTGTC-3′	125
		A: 5′-CGTTGTTGTGGCAGTTGTTGGT-3′	
PAT2	XM_005672617	S: 5′-GGGCTACTTGCGGTTCGG-3′	181
		A: 5′-GCGCTTTGACACCTGGGAG-3′	
LAT1	NM_001110421	S: 5′-TTTGTTATGCGGAACTGG-3′	155
		A: 5′-AAAGGTGATGGCAATGAC-3′	
ASCT2	XM_003127238	S: 5′-GATTGTGGAGATGGAGGATGTGG-3′	128
		A: 5′-TGCGAGTGAAGAGGAAGTAGATGA-3′	
SNAT2	XM_005664159	S: 5′-TACTTGGTTCTGCTGGTGTCC-3′	212
		A: 5′-GTTGTGGGCTGTGTAAAGGTG-3′	
$b^{0,+}$	EU390780	S: 5′-GAACCCAAGACCACAAATC-3′	180
		A: 5′-ACCCAGTGTCGCAAGAAT-3′	
EAAC1	NM_001164649	S: 5′-GGCACCGCACTCTACGAAGCA-3′	177
		A: 5′-GCCCACGGCACTTAGCACGA-3′	
PepT1	NM_214347	S: 5′-CATCGCCATACCCTTCTG-3′	143
		A: 5′-TTCCCATCCATCGTGACATT-3′	
GAPDH	NM_001206359	S: 5′-AAGGAGTAAGAGCCCCTGGA-3′	140
		A: 5′-TCTGGGATGGAAACTGGAA-3′	

T1R1/T1R3, taste receptor type 1 member 1/member 3; mTOR, mammalian target of rapamycin; PAT1 and PAT2, proton-assisted AA transporters; LAT1, L-type AA transporter 1; ASCT2, ASC-like Na^+-dependent neutral AA transporter 2; SNAT2, sodium-coupled neutral AA transporter 2; $b^{0,+}$, $b^{0,+}$ AA transporter; EAAC1, excitatory AA carrier 1; PepT1, H^+/peptide co-transporter; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

Cary, NC, USA), and by comparing means with Tukey's method. The effects of pig strain, dietary protein level, physiological stage, and their interactions were all taken into account. Log transformation of variables was performed when variance of data was not homogenous among treatment groups, as assessed using the Levene's test (Wei et al. 2012). Results are presented with means plus pooled SEM. Effects were considered statistically significant at $P < 0.05$. Probability values between 0.05 and 0.10 were considered to be trends.

Results

Plasma concentrations of free amino acids

As shown in Table 5, the concentrations of most AAs in the plasma were affected by the developmental stages of the pigs. As the age increased, the concentrations of Ala, Arg, Asn, Asp, Glu, Gly, Ile, Orn, Pro, Ser, Tau, and Tyr gradually decreased ($P < 0.05$). Plasma concentrations of 3-methylhistidine (3 M His), Cys, Gln, His, Phe, and Thr were much higher in the nursery phase ($P < 0.05$) than in the growing and finishing phases, but the concentration of 1 M His was lower ($P < 0.05$) in the nursery stage as compared to the growing and finishing phases. In contrast, the plasma concentration of α-aminobutyric acid was greater ($P < 0.05$) in the finishing phase than in the other two phases.

When compared to Landrace pigs, Bama mini-pigs had higher ($P < 0.05$) plasma concentrations of 3 M His, α-aminoadipic acid, Ile, and Val, and lower ($P < 0.05$) concentrations of Asp throughout the trial. In addition, Bama mini-pigs had higher ($P < 0.05$) plasma concentrations of Leu and Trp and a lower ($P < 0.05$) concentration of Cys in the nursery phase. They also had lower ($P < 0.05$) plasma

concentrations of Ala, Cys, Gly, Met, Orn, and Ser in the growing phase, and lower ($P < 0.05$) concentrations of Ala, Gly, Met, and Ser but a higher ($P < 0.05$) concentration of Trp in the finishing phase, as compared to Landrace pigs.

Overall, the NRC diet increased ($P < 0.05$) plasma concentrations of 1 M His, 3 M His (except for Landrace pigs in the finishing phase), α-aminoadipic acid, and α-aminobutyric acid, as compared with the GB diet. When pigs were fed the GB diet, plasma concentrations of Gly increased ($P < 0.05$), especially in the finishing phase. Phase × strain interactions were observed for most AAs, notably 1 M His, Ala, Asn, Cys, Gly, His, Ile, Leu, Lys, Met, Orn, Pro, Ser, Tau, Trp, Tyr, and Val. Diet type interacted with the developmental phase for the plasma concentrations of α-aminobutyric acid, Cys, Orn, and Trp, and with the pig strain for the concentrations of Asn, Lys, Orn, Ser, Trp, and Tyr. No interactions among phase, strain, and diet were observed for any AA.

Free amino acid pools in *longissimus dorsi* muscle

The concentrations of free AAs in LDM are shown in Table 6. The concentrations of Ala, Asp, Glu, Gly, Orn, Pro, and Tyr in both strains of pigs decreased ($P < 0.05$) over time, while those of carnosine and anserine increased ($P < 0.05$). Most AAs decreased ($P < 0.05$) during the growing phase (as compared to the nursery phase), but increased ($P < 0.05$) at the finishing phase, including α-aminoadipic acid, Arg, GABA, Gln, Ile, Lys, Met, Phe, Ser, Thr, and Val. In contrast, as compared to the nursery phase, the concentrations of 3 M His and Asn increased ($P < 0.05$) during the growing phase and decreased ($P < 0.05$) in the finishing phase.

We found that pig strain affected ($P < 0.05$) the concentrations of free AAs in LDM. More specifically, Landrace pigs had a higher ($P < 0.05$) intramuscular Ala concentration but lower ($P < 0.05$) intramuscular 3 M His concentration than Bama mini-pigs throughout the trial. Landrace pigs also had higher ($P < 0.05$) intramuscular concentrations of Arg, Asp, Glu, Leu, Lys, Met, Phe, Ser, Thr, and Tyr in the nursery phase, of Leu and Phe in the growing phase, and of Arg in the finishing phase. Furthermore, the responses of intramuscular AA profiles to the different dietary levels of protein were dependent on pig strain.

Pigs eating the GB diet had increased ($P < 0.05$) α-aminoadipic acid concentration in their LDM, as compared to those eating the NRC food. The Bama mini-pigs eating the GB diet also had a greater ($P < 0.05$) carnosine concentration in their LDM during the growing and finishing phases. In contrast, the Gln concentrations in the LDM of Landrace pigs consuming the GB diet during the growing and finishing phases were lower ($P < 0.05$) than those of pigs eating the NRC diet.

Free amino acid pools in *biceps femoris* muscle

In BFM, the concentrations of 3 M His, anserine, and carnosine increased ($P < 0.05$) gradually, while those of Ala, Arg, Asn, Glu, Gly, Orn, and Pro decreased ($P < 0.05$) with increasing age (Table 7). The intramuscular concentrations of Asp, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr, and Val in the nursery phase were higher ($P < 0.05$) than those in the growing and finishing phases.

Landrace pigs had higher ($P < 0.05$) concentrations of β-Ala, GABA, and Phe, and lower concentrations of anserine and 3 M His in BFM ($P < 0.05$) than Bama mini-pigs throughout the trial. In addition, as compared to Bama mini-pigs, Landrace pigs had higher ($P < 0.05$) intramuscular concentrations of Ala, Arg, Leu, Asn, Gly, Lys, Met, Pro, Ser, Thr, and Tyr in the nursery phase, of Asn, Gly, Met, and Pro in the growing phase, and of Tyr in the finishing phase.

Overall, the contrasting protein contents of the NRC and GB diets affected intramuscular AA pools in the two strains of pigs differently. However, the NRC diet increased ($P < 0.05$) intramuscular His concentration regardless of pig strain, as well as the intramuscular concentrations of Ala and Gly in both strains of pigs (except for Bama mini-pigs in the finishing phase).

mRNA levels for AA-sensing genes in muscle

As shown in Table 8, the mRNA levels for Calpain-3 in the LDM decreased ($P < 0.05$) as age increased. The mRNA level for mTOR increased ($P < 0.05$) in the growing phase, but decreased ($P < 0.05$) in the finishing phase, as compared to the nursery phase. In the LDM of Bama mini-pigs, the mRNA level for mTOR was higher ($P < 0.05$), while that for calpain-3 (especially in the growing and finishing phases) was lower ($P < 0.05$) than in Landrace pigs. A strain × phase interaction was observed ($P < 0.05$) for the mRNA level of Calpain-3.

The mRNA level for mTOR in BFM increased ($P < 0.05$) over time. Landrace pigs had higher ($P < 0.05$) mRNA levels in BFM associated with calpain-3 and T1R3 (especially in the nursery and finishing phases) than Bama mini-pigs. Furthermore, the NRC diet enhanced ($P < 0.05$) the mRNA level for calpain-3 but reduced ($P < 0.05$) that for T1R3 in BFM, when compared to the GB diet. Strain × phase interactions were observed ($P < 0.05$) for mRNA levels for T1R1 and T1R3 in BFM.

mRNA levels for AA and peptide transporters in muscle

Table 9 shows that, with advancing age, SNAT2 mRNA levels in skeletal muscle increased ($P < 0.05$), while $b^{0,+}$ transporter mRNA levels decreased ($P < 0.05$). Compared to the

Table 5 Plasma concentrations of free AAs in pigs

Items	Nursery phase				Growing phase				Finishing phase				SEM	*P* values						
	Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig			P_P	P_S	P_{P*S}	P_D	P_{P*D}	P_{S*D}	P_{P*S*D}
	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet								
1 M His	0.59	0.90	0.35	0.86	0.68	1.15	1.23	1.26	1.10	1.03	1.15	1.17	0.12	<0.01	0.21	0.03	0.01	0.07	0.74	0.18
3 M His	1.83	2.36	3.50	4.52	1.16	1.72	3.27	3.78	1.88	1.55	3.20	3.56	0.29	0.01	<0.01	0.63	0.01	0.21	0.29	0.69
α-Aminoadipic acid	2.38	2.46	5.34	6.69	1.61	4.15	5.87	5.67	3.51	6.98	4.75	6.22	0.94	0.22	<0.01	0.06	0.01	0.46	0.32	0.30
α-Aminobutyric acid	0.61	0.85	1.25	1.10	0.54	1.02	0.81	1.29	0.93	2.25	0.82	2.35	0.27	<0.01	0.16	0.56	<0.01	<0.01	0.86	0.75
Ala	57.08	55.92	67.25	53.91	51.55	63.46	27.88	34.91	28.78	39.85	34.00	28.14	5.10	<0.01	<0.01	<0.01	0.61	0.08	0.07	0.74
Arg	30.46	29.00	31.91	29.15	19.94	28.19	18.71	19.03	19.31	22.06	16.39	14.40	0.10	<0.01	0.10	0.32	0.66	0.38	0.23	0.77
Asn	13.26	13.30	14.97	12.24	7.84	13.02	5.85	5.45	5.14	6.45	6.92	5.13	1.41	<0.01	0.11	0.03	0.76	0.19	0.03	0.76
Asp	4.92	3.83	4.03	3.51	3.35	4.56	2.77	3.16	2.73	2.13	2.13	1.86	0.51	<0.01	0.03	0.76	0.64	0.08	0.97	0.61
Cys	6.41	5.69	4.53	2.96	2.28	2.87	1.46	2.61	2.33	1.89	2.10	2.07	0.53	<0.01	<0.01	0.01	0.60	0.04	0.94	0.61
Gln	60.92	73.77	81.12	71.92	62.75	65.61	56.14	59.28	62.98	58.97	68.88	55.26	5.47	0.01	0.71	0.15	0.69	0.31	0.12	0.38
Glu	52.16	42.04	49.89	47.73	36.47	42.19	38.14	36.18	27.00	22.18	31.64	23.92	3.57	<0.01	0.68	0.60	0.11	0.22	0.84	0.31
Gly	68.76	66.61	82.13	67.78	79.25	79.02	52.04	53.79	67.28	54.86	68.75	42.67	5.67	0.01	0.02	<0.01	0.01	0.08	0.26	0.60
His	25.54	23.87	30.25	30.43	23.76	27.25	22.69	20.47	21.98	23.06	26.59	21.20	2.14	0.01	0.44	0.01	0.57	0.70	0.19	0.34
Ile	16.73	14.99	26.22	25.90	15.39	19.61	21.09	20.17	14.00	17.71	18.82	18.19	1.90	0.03	<0.01	0.01	0.54	0.55	0.25	0.43
Leu	26.78	24.73	39.03	38.44	27.32	31.74	30.96	29.30	23.75	28.91	29.82	25.93	2.85	0.06	<0.01	<0.01	0.90	0.80	0.20	0.44
Lys	23.37	27.00	41.50	34.69	20.16	37.38	23.02	24.44	28.41	34.95	24.56	22.57	3.68	0.12	0.97	<0.01	0.14	0.13	0.01	0.80
Met	5.96	5.63	6.58	5.81	5.29	7.33	4.14	4.12	5.12	5.59	4.97	4.43	0.57	0.06	0.02	0.01	0.68	0.18	0.10	0.62
Orn	21.55	18.59	24.86	17.61	15.81	23.49	8.41	8.47	7.42	8.16	8.03	6.51	1.40	<0.01	<0.01	<0.01	0.53	<0.01	0.01	0.46
Phe	17.46	16.01	19.40	17.37	12.63	16.58	14.37	14.46	12.87	16.74	15.17	14.80	1.20	<0.01	0.46	0.54	0.36	0.06	0.05	0.52
Pro	40.95	38.43	53.15	48.55	38.28	44.92	28.48	25.85	23.76	23.49	37.20	23.22	4.68	<0.01	0.70	<0.01	0.32	0.44	0.15	0.70
Ser	21.12	21.48	24.50	20.33	18.54	23.72	12.22	12.26	13.98	16.26	12.99	10.73	1.68	<0.01	<0.01	<0.01	0.82	0.19	0.02	0.99
Tau	25.67	29.59	38.35	34.05	28.77	26.13	22.30	24.37	20.79	15.73	17.06	12.72	2.45	<0.01	0.81	<0.01	0.26	0.40	0.76	0.18
Thr	33.01	30.47	39.61	29.10	18.49	25.37	17.26	16.73	16.20	19.95	18.99	19.39	2.97	<0.01	0.83	0.20	0.82	0.05	0.09	0.86
Trp	10.70	8.86	13.85	11.87	8.29	12.35	10.74	9.34	8.83	11.23	10.93	10.59	0.86	0.16	0.03	0.03	0.78	0.02	0.01	0.11
Tyr	14.80	15.42	23.25	19.59	13.99	16.97	11.35	11.24	10.16	13.10	11.64	10.10	1.32	<0.01	0.58	<0.01	0.80	0.28	0.02	0.93
Val	35.64	30.93	51.90	53.00	31.89	38.26	47.78	44.39	32.85	39.99	44.05	40.44	3.54	0.40	<0.01	0.04	0.83	0.75	0.26	0.21

Values, expressed as μg/ml, are means plus pooled SEM, $n = 8$ per treatment group

P phase, *S* strain, *P* × *S* phase × strain interaction, *D* diet, *P* × *D* phase × diet interaction, *S* × *D* strain × diet interaction, *P* × *S* × *D* phase × strain × diet interaction

Table 6 Concentrations of free amino acids in *longissimus dorsi* muscle of pigs after dietary intervention

Items	Nursery phase				Growing phase				Finishing phase				SEM	*P* values						
	Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig			P_P	P_S	P_{P*S}	P_D	P_{P*D}	P_{S*D}	P_{P*S*D}
	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet								
3 M His	1.70	1.94	2.66	2.13	1.37	2.25	2.99	2.70	1.83	1.64	1.64	3.24	0.31	<0.01	<0.01	<0.01	0.38	0.01	0.04	<0.01
α-Aminoadipic acid	11.97	8.79	8.94	6.86	4.94	4.45	5.11	4.73	7.60	4.25	7.36	2.83	0.88	<0.01	0.14	0.27	<0.01	0.14	1.00	0.78
Ala	145.34	153.71	100.05	87.59	68.25	97.89	89.78	53.64	68.48	61.59	58.90	47.16	6.54	<0.01	<0.01	<0.01	0.34	0.82	<0.01	0.06
Anserine	25.77	19.50	14.89	12.24	13.66	17.03	26.10	64.02	90.87	77.07	75.36	52.69	6.96	<0.01	0.57	<0.01	0.46	<0.01	0.14	0.50
Arg	51.48	44.47	8.50	13.67	2.88	1.81	3.04	3.11	7.11	7.65	5.57	3.98	2.41	<0.01	<0.01	<0.01	0.73	0.99	0.33	0.25
Asp	40.56	36.62	14.36	15.60	28.90	36.47	30.73	19.02	6.09	2.68	6.98	3.63	3.06	<0.01	<0.01	<0.01	0.66	0.15	0.02	<0.01
Asn	14.96	12.99	13.13	15.58	19.28	35.84	26.43	24.36	4.42	4.63	4.54	5.36	2.05	<0.01	0.78	0.77	0.10	0.16	0.16	0.01
β-Ala	30.56	28.96	29.38	22.29	17.06	25.38	18.40	12.49	21.97	22.96	17.73	14.97	1.98	<0.01	0.02	0.40	0.84	0.03	<0.01	0.02
Carnosine	1555.93	1840.13	2066.40	2098.97	2157.44	1907.23	2326.98	2365.83	2992.77	3135.72	2990.34	2645.68	150.59	<0.01	<0.01	<0.01	<0.01	<0.01	0.44	<0.01
Ethanol amine	0.94	1.88	2.03	1.38	1.10	1.72	2.49	1.52	2.31	2.95	1.18	1.28	0.24	0.25	0.36	<0.01	0.54	0.52	<0.01	0.42
GABA	1.59	1.71	1.08	0.88	1.32	0.69	1.23	1.08	1.49	1.18	2.12	1.74	0.18	0.01	0.85	<0.01	0.07	0.53	0.92	0.51
Glu	53.86	34.67	9.72	29.10	35.28	46.68	42.86	24.65	13.04	10.37	13.57	6.09	2.53	<0.01	<0.01	<0.01	0.27	<0.01	0.03	<0.01
Gln	185.83	190.21	216.98	172.60	105.61	59.54	66.43	101.67	198.55	131.49	111.32	133.44	13.74	<0.01	0.10	0.16	0.04	0.98	<0.01	<0.01
Gly	83.79	112.08	81.50	94.12	35.51	34.57	69.44	31.03	32.60	33.61	34.02	27.67	6.62	<0.01	0.86	0.14	0.90	0.01	0.06	0.50
His	8.22	7.83	7.83	2.00	3.50	5.75	6.00	5.51	4.83	4.78	8.22	5.23	0.87	0.31	0.98	<0.01	0.07	0.07	<0.01	0.65
Ile	29.56	26.48	13.09	15.22	3.37	3.13	6.43	14.52	11.51	11.48	15.15	11.34	1.77	<0.01	0.25	<0.01	0.71	0.23	0.25	0.20
Leu	60.27	49.68	17.69	18.56	9.63	32.24	9.42	14.56	16.44	14.76	19.01	15.22	2.86	<0.01	<0.01	<0.01	0.35	<0.01	0.55	0.04
Lys	102.63	70.76	8.73	14.08	2.27	1.24	1.48	7.19	15.83	14.23	15.67	8.63	3.95	<0.01	<0.01	<0.01	0.11	0.13	0.04	0.02
Met	27.02	24.37	17.17	11.09	4.78	2.50	2.88	3.18	5.59	5.81	6.20	4.54	1.54	<0.01	<0.01	<0.01	0.10	0.37	0.71	0.59
Orn	14.35	11.06	7.37	7.59	4.66	6.27	5.95	13.21	3.46	3.20	2.91	1.22	0.87	<0.01	0.25	<0.01	0.35	<0.01	0.06	0.11
Phe	37.47	30.26	21.95	12.25	3.99	7.61	2.84	6.81	11.95	12.88	13.03	8.22	2.06	<0.01	<0.01	<0.01	0.18	0.01	0.42	0.75
Pro	94.79	153.30	103.59	129.26	70.51	80.35	91.17	14.93	9.51	9.83	12.35	16.48	7.91	<0.01	0.18	0.23	0.55	<0.01	<0.01	0.02
Ser	47.60	38.96	2.38	2.53	1.67	5.90	5.15	5.21	8.37	9.02	9.29	8.48	1.32	<0.01	<0.01	<0.01	0.49	0.04	0.61	0.03
Thr	37.52	37.98	12.84	11.19	6.62	7.79	5.03	6.61	8.40	10.80	11.53	10.30	1.51	<0.01	<0.01	<0.01	0.70	0.79	0.45	0.79
Tyr	37.13	30.04	11.72	11.63	8.92	26.88	8.51	3.22	5.83	6.20	8.46	3.62	2.05	<0.01	<0.01	<0.01	0.92	0.04	0.03	<0.01
Val	34.70	33.88	29.36	21.79	5.65	13.24	5.49	12.21	17.13	14.70	17.99	14.48	2.82	<0.01	0.18	0.17	1.00	0.09	0.51	0.82

Values, expressed as mg AA/100 g freeze-dried muscle tissue, are means plus pooled SEM, $n = 8$ per treatment group

P phase, *S* strain, *P* × *S* phase × strain interaction, *D* diet, *P* × *D* phase × diet interaction, *S* × *D* strain × diet interaction, *P* × *S* × *D* phase × strain × diet interaction

Table 7 Concentrations of free amino acids and dipeptides in *biceps femoris* muscle of pigs after dietary intervention

Items	Nursery phase				Growing phase				Finishing phase				SEM	*P* values						
	Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig			P_P	P_S	P_{P*S}	P_D	P_{P*D}	P_{S*D}	P_{P*S*D}
	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet								
3 M His	1.72	1.33	1.86	2.15	2.07	2.57	2.29	2.00	2.24	1.74	2.54	4.27	0.20	<0.01	<0.01	<0.01	0.15	0.19	0.02	<0.01
α-Aminoadipic acid	11.90	11.12	12.93	13.50	10.32	18.02	14.33	18.51	14.79	11.08	7.81	7.43	0.74	<0.01	0.42	<0.01	0.03	<0.01	0.73	0.05
Ala	195.39	244.88	153.32	178.49	96.92	111.99	98.95	118.50	68.49	100.41	90.04	83.19	9.70	<0.01	0.03	<0.01	<0.01	0.34	0.19	0.48
Anserine	41.06	46.21	55.35	70.22	86.53	61.70	91.85	80.89	68.81	96.12	138.53	127.54	5.75	<0.01	<0.01	<0.01	0.98	0.02	0.57	0.03
Arg	59.47	48.91	12.87	19.15	8.39	12.14	13.55	14.75	8.64	10.15	9.16	7.77	2.22	<0.01	<0.01	<0.01	0.94	0.52	0.26	0.03
Asp	28.72	29.97	21.66	21.12	6.06	8.29	6.71	8.78	4.20	5.88	9.43	10.63	1.76	<0.01	0.55	<0.01	0.33	0.85	0.76	0.96
Asn	37.87	30.26	6.27	15.07	8.37	8.42	6.64	6.44	4.56	6.04	6.21	5.35	1.85	<0.01	<0.01	<0.01	0.84	0.98	0.11	0.01
β-Ala	41.00	30.80	23.37	18.22	27.54	32.95	9.29	12.25	24.75	33.74	21.62	21.17	1.95	<0.01	<0.01	<0.01	0.86	<0.01	0.44	0.14
Carnosine	1458.79	925.34	1438.59	1601.56	2219.34	1622.31	1763.10	1765.84	2079.94	2257.47	2272.73	2397.56	82.52	<0.01	0.08	<0.01	0.08	0.02	<0.01	0.04
Ethanol amine	2.88	1.74	2.44	2.02	1.51	1.40	2.22	3.04	3.43	2.83	3.05	4.32	0.37	<0.01	0.06	0.18	0.92	0.17	0.04	0.68
GABA	2.51	2.37	1.13	1.45	2.13	1.59	1.60	1.77	1.62	1.48	1.51	1.00	0.22	0.07	<0.01	0.04	0.40	0.59	0.43	0.40
Glu	61.04	69.65	42.72	39.38	14.72	17.77	23.37	27.11	13.68	17.13	26.77	16.87	4.23	<0.01	0.36	<0.01	0.77	0.66	0.20	0.62
Gln	280.94	181.90	264.13	249.98	157.35	225.23	211.81	167.58	182.25	139.41	210.92	129.80	355.99	<0.01	0.44	<0.01	0.58	0.45	0.27	0.31
Gly	102.35	110.94	85.84	102.44	64.77	71.80	40.70	58.42	41.27	49.93	54.28	48.78	4.81	<0.01	0.02	0.02	0.02	0.38	0.84	0.33
His	20.19	25.78	16.46	21.60	13.00	11.35	13.29	15.37	11.33	12.39	16.21	16.64	1.28	<0.01	0.35	<0.01	0.03	0.06	0.65	0.58
Ile	31.87	29.53	12.27	15.62	7.74	10.64	11.17	10.92	8.54	12.89	10.73	10.14	1.42	<0.01	<0.01	<0.01	0.25	0.87	0.71	0.10
Leu	60.60	54.25	18.77	23.27	11.88	15.66	14.43	13.19	11.97	18.73	15.22	13.80	2.05	<0.01	<0.01	<0.01	0.52	0.63	0.80	0.03
Lys	38.47	45.60	22.52	24.23	10.12	14.61	15.41	16.07	13.47	16.28	12.26	11.63	1.95	<0.01	<0.01	<0.01	0.07	0.65	0.16	0.96
Met	26.49	22.85	5.46	10.31	7.35	4.66	3.05	2.66	5.65	7.95	8.19	7.96	1.60	<0.01	<0.01	<0.01	0.98	0.65	0.26	0.18
Orn	14.63	12.38	7.42	9.86	7.59	5.90	4.37	4.15	4.86	3.19	3.04	2.86	0.96	<0.01	<0.01	0.11	0.42	0.79	0.09	0.58
Phe	37.70	34.03	10.28	14.94	10.42	12.57	9.83	10.11	12.13	14.92	11.23	11.24	1.79	<0.01	<0.01	<0.01	0.45	0.96	0.65	0.18
Pro	38.69	39.01	21.37	31.28	29.67	17.83	10.49	10.70	8.89	11.37	14.10	10.31	2.57	<0.01	<0.01	<0.01	0.82	0.08	0.19	0.13
Ser	55.28	49.04	16.68	22.94	10.69	13.99	12.29	12.91	10.16	13.33	11.65	10.63	2.55	<0.01	<0.01	<0.01	0.60	0.91	0.63	0.15
Thr	41.30	44.07	20.48	21.09	10.98	14.40	13.02	13.05	10.84	13.53	10.67	12.55	1.86	<0.01	<0.01	<0.01	0.18	0.98	0.45	0.93
Tyr	35.71	33.54	10.84	13.80	8.07	11.07	12.15	10.96	13.63	14.34	8.80	7.74	2.16	<0.01	<0.01	<0.01	0.82	0.97	0.93	0.47
Val	37.73	36.25	19.30	27.56	16.32	14.56	18.21	16.41	11.78	19.21	20.93	21.08	1.83	<0.01	0.14	<0.01	0.20	0.20	0.77	0.05

Values, expressed as mg AA/100 g freeze-dried muscle tissue, are means plus pooled SEM, n = 8 per treatment group

P phase, *S* strain, *P* × *S* phase × strain interaction, *D* diet, *P* × *D* phase × diet interaction, *S* × *D* strain × diet interaction, *P* × *S* × *D* phase × strain × diet interaction

nursery phase, the PAT1 mRNA level increased ($P < 0.05$) during the growing phase and decreased ($P < 0.05$) in the finishing phase. In contrast, the mRNA levels for PAT2 and transporter EAAC1 decreased ($P < 0.05$) during the growing phase and increased ($P < 0.05$) in the finishing phase, when compared to the nursery phase. When strain differences were compared, the mRNA levels for SNAT2, EAAC1, and PAT1 in LDM were higher ($P < 0.05$), while those for the oligopeptide transporter PepT1 and AA transporter $b^{0,+}$ were lower ($P < 0.05$) in Bama mini-pigs than in Landrace pigs. All pigs fed the NRC diet had higher ($P < 0.05$) mRNA levels for PAT1, LAT1, and PepT1 than those fed the GB diet. A strain × diet interaction ($P < 0.05$) was observed for the mRNA level of PAT2, and strain × phase interactions ($P < 0.05$) were observed for SNAT2, $b^{0,+}$, and EAAC1 mRNA levels in LDM.

As shown in Table 10, the mRNA levels for $b^{0,+}$ and PepT1 transporters in BFM increased ($P < 0.05$) with age. The mRNA levels for PAT1 and SNAT2 increased ($P < 0.05$) during the growing phase and decreased ($P < 0.05$) in the finishing phase, when compared to the nursery phase. The mRNA levels for LAT1 and ASCT2 decreased ($P < 0.05$) in the growing phase and increased ($P < 0.05$) in the finishing phase, when compared to the nursery phase. In contrast to Landrace pigs, Bama mini-pigs had higher ($P < 0.05$) mRNA levels for LAT1 and ASCT2 in the nursery and finishing phases, as well as mRNA levels for PepT1 in nursery and growing phases, and lower ($P < 0.05$) mRNA levels for PepT1 in the finishing phase. The NRC diet enhanced ($P < 0.05$) the mRNA levels for LAT1 and ASCT2 to a greater extent than the GB diet. Strain × diet interactions ($P < 0.05$) were observed for mRNA levels corresponding to LAT1 and $b^{0,+}$, and strain × phase interactions ($P < 0.05$) were observed for mRNA levels corresponding to ASCT2, SNAT2, and PepT1 in BFM.

Discussion

The small intestine is a major site of AA catabolism in humans and animals (Swaid et al. 2013; Wang et al. 2008). In this organ, enterocytes utilize AAs for ATP production, protein synthesis, and generation of various metabolites that exert physiological effects locally and in peripheral tissues (Blachier et al. 2013). Intestinal metabolism plays an important role in the entry of dietary AAs into the portal circulation as well as the plasma pattern of AAs (Riedijk et al. 2007; Wu 1998). In pigs, the capacity of the intestines to catabolize AAs can vary with age and the time post feeding, which results in fluctuating AA concentrations in the portal blood over time (Blachier et al. 2013). In the present study, the concentrations of plasma AAs were the highest during the nursery phase for both pig strains, regardless of diet, and then declined markedly as age increased.

Plasma AA concentrations are affected by a variety of factors, including intracellular protein synthesis and degradation in tissues, AA transport and metabolism, and intestinal microbiota activity (Blachier et al. 2007; Dai et al. 2011, 2012, 2013). There is evidence that the pig small intestine extensively catabolizes AA in a segment-dependent manner (Dai et al. 2015; Yang et al. 2014) such that nearly 50 % of total dietary AA (the sum of all AA) do not enter the portal circulation. Based on results of the present study, we suggest that an overall increase in AA catabolism in the pig small intestine of occurs with age. Substantial differences in several plasma AAs were observed between Bama mini-pigs and Landrace pigs. The plasma concentrations of the branched-chain amino acids (BCAAs), namely Ile, Leu, and Val, in Landrace pigs were much lower than those in Bama mini-pigs, especially during the nursery and finishing phases. The decrease in the circulating concentrations of AAs in Landrace pigs may be secondary to increased catabolism in the small intestine, skeletal muscle, and other tissues, since BCAAs provide α-amino groups for the endogenous synthesis of glutamine, especially in skeletal muscle (Wu 2009; Yoneda et al. 2009). In addition, Leu activates the Ser/Thr protein kinase mTOR signaling pathway that upregulates protein synthesis and cell growth (Duan et al. 2015). We also found that Bama mini-pigs had higher concentrations of 3 M His than Landrace pigs throughout the trial. In some species such as cattle (Houweling et al. 2012), an increase in the circulating concentration of 3 M His is a useful indicator of muscle protein degradation. If this is also true for growing swine, our findings suggest that Bama mini-pigs may have a greater rate of muscle proteolysis and a lower rate of AA deposition in muscule protein. Further studies are needed to test this hypothesis.

Concentrations of free AA in tissues reflect the nutritional status of an animal (He et al. 2012; Sales et al. 2013), as protein synthesis is regulated by intracellular AA concentration (Miyazaki and Esser 2009). In addition, free AAs are essential for tissue growth because they regulate protein synthesis and catabolism to favor net protein deposition in tissues, especially skeletal muscle (Burrin et al. 1995). In the present study, concentrations of most free AA in the muscles of Landrace pigs were higher than those in Bama mini-pigs, which may contribute to dynamic protein turnover and muscle growth in this lean pig strain.

Biological sensing of AA in vivo plays a key role in coupling changes in whole-body protein and AA metabolism, which allows appropriate physiological responses. Receptors for umami taste and sweet taste are closely related to each other (San Gabriel and Uneyama 2013). The umami taste receptor T1R1/T1R3 mediates the response to umami

Table 8 mRNA levels for AA-sensing genes in skeletal muscles of growing-finishing pigs

Items	Nursery phase		Growing phase				Finishing phase				SEM	*P* values					
	Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig			P_P	P_S	P_{P*S}	P_D	P_{P*D}	P_{S*D}	P_{P*S*D}
	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet								
Longissimus dorsi muscle																				
T1R1	2.02	2.37	2.00	1.74	0.66	1.70	1.08	1.70	1.34	2.11	2.12	1.41	0.40	0.05	0.92	0.66	0.24	0.36	0.11	0.70
T1R3	1.00	1.01	1.10	1.14	1.78	1.35	2.02	1.27	1.34	1.21	1.41	1.33	0.29	0.05	0.61	1.00	0.24	0.35	0.83	0.91
Calpain-3	3.15	2.46	2.30	3.38	2.69	2.75	1.07	0.92	2.05	1.35	0.63	0.41	0.36	<0.01	0.01	<0.01	0.66	0.53	0.15	0.19
mTOR	0.91	0.58	0.77	1.04	0.55	1.95	2.13	1.87	1.18	1.20	1.50	1.57	0.22	<0.01	<0.01	0.21	0.17	0.16	0.24	<0.01
Biceps femoris muscle																				
T1R1	2.12	1.47	1.59	0.84	0.74	2.04	2.28	1.13	1.22	0.59	1.05	1.66	0.27	0.12	0.71	0.03	0.22	0.12	0.21	<0.01
T1R3	2.31	1.42	1.38	0.76	2.63	1.04	2.46	1.71	2.24	1.74	1.64	1.08	0.27	0.06	0.02	0.03	<0.01	0.32	0.30	0.56
Calpain-3	2.84	2.75	0.25	0.77	2.56	3.07	0.17	0.21	1.94	2.83	0.04	0.69	0.26	0.39	<0.01	0.35	0.01	0.34	0.92	0.36
mTOR	0.73	0.50	1.35	1.00	1.04	2.04	1.33	1.39	1.44	1.33	2.49	1.59	0.33	<0.01	0.10	0.21	0.67	0.11	0.14	0.68

Values are means plus pooled SEM, $n = 8$ per treatment group

The mRNA levels for T1R1 (taste receptor type 1 member 1), T1R3 (taste receptor type 1 member 3), calpain-3 and mTOR (mammalian target of rapamycin) were normalized using GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as an internal control

P phase, *S* strain, $P \times S$ phase × strain interaction, *D* diet, $P \times D$ phase × diet interaction, $S \times D$ strain × diet interaction, $P \times S \times D$ phase × strain × diet interaction

ligands, such as monosodium glutamate. In the present study, T1R1/T1R3 expression was higher in the BFM muscle of pigs fed the GB diet than in those fed the NRC diet, indicating that a low-protein diet upregulates the gene expression of the umami taste receptor. In addition, pig genotype interacted with the developmental stage regarding the mRNA levels corresponding to T1R1/T1R3 receptors. It remains to be determined whether changes in mRNA levels for these receptors can be translated into changes in their protein abundances.

Previous studies have indicated that AA-induced activation of mTORC1 is developmentally regulated in skeletal muscle (Suryawan and Davis 2010; Suryawan et al. 2013), and indeed the present study shows that as age increased, the expression of mTOR in BFM also increased. Additionally, expression of mTOR in LDM was higher in the growing phase but lower in the finishing phase. The calpain system plays an important role in myofibrillar protein degradation. Muscle growth and postmortem tenderization of meat are highly related to the degree of proteolysis, and therefore, the calpain system activity affects muscle growth and meat tenderness (Tait et al. 2014). The decreased expression of calpain-3 with increasing age might indicate a lower level of tenderness as the animals growing. Results of this investigation showed that the calpain-3 mRNA level in Landrace pigs was higher than that in Bama mini-pigs, suggesting improved meat tenderness in the Landrace strain.

Recent studies have indicated that AA transporters not only act as nutrient transporters, but also as nutrient signaling components responsible for the activation of mTORC1, which activates protein translation (Heublein et al. 2010; Nicklin et al. 2009; Pinilla et al. 2011). Our results showed that the strain of pig affected the mRNA levels for AA transporters. In particular, the mRNA levels for SNAT2, EAAC1, and PAT1 in LDM, and LAT1 and ASCT2 in BFM were higher, while the mRNA levels for PepT1 (especially in the finishing phase) were lower in Bama mini-pigs, compared with Landrace pigs. In addition, an increasing number of studies have demonstrated that large neutral AAs (BCAAs and aromatic AAs) in the plasma are taken up by muscle cells via the large neutral AA transporter LAT1 (Suryawan et al. 2013), which is crucial for platelet-derived growth factor-induced vascular smooth muscle growth (Liu et al. 2004). SNAT2 transports glutamine into the cell for the LAT1-CD98 bi-transport system to export Gln and increase the influx of large neutral AA such as Leu (Baird et al. 2009). Both LAT1 and SNAT2 are related to the activation of the mTOR signaling pathway (Nicklin et al. 2009; Pinilla et al. 2011). EAAC1 is also a key transporter for glutamate (Fu et al. 2013). Upregulation of muscular AA transporters allows for greater uptake and accumulation of AAs in muscle tissue and, therefore, enhances lean protein

Table 9 mRNA levels for AA and peptide transporters in *longissimus dorsi* muscle of growing-finishing pigs

Items	Nursery phase				Growing phase				Finishing phase				SEM	*P* values						
	Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig			P_P	P_S	P_{P*S}	P_D	P_{P*D}	P_{S*D}	P_{P*S*D}
	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet								
PAT1	0.71	0.68	1.00	1.11	0.76	1.49	1.53	1.80	1.18	1.29	1.14	1.35	0.15	<0.01	<0.01	0.11	0.02	0.12	0.71	0.37
PAT2	1.48	0.97	1.32	1.19	0.85	0.89	1.16	1.85	2.00	1.71	1.36	1.96	0.22	<0.01	0.26	0.05	0.64	0.10	0.02	0.76
LAT1	1.15	1.08	1.30	1.38	0.74	2.05	0.97	1.19	1.04	1.42	0.62	1.15	0.22	0.54	0.31	0.15	<0.01	0.07	0.35	0.11
ASCT2	1.52	1.15	1.23	0.99	0.74	1.12	1.10	1.02	0.97	1.37	0.96	1.35	0.17	0.20	0.74	0.38	0.46	0.03	0.59	0.48
SNAT2	0.64	0.52	1.12	0.86	1.21	0.98	1.43	1.65	1.40	1.05	2.74	2.77	0.16	<0.01	<0.01	<0.01	0.27	0.74	0.28	0.42
$b^{0,+}$	3.35	3.08	1.45	2.11	1.65	1.32	2.04	1.00	1.63	1.26	0.33	1.56	0.31	<0.01	<0.01	<0.01	0.92	0.07	0.14	0.07
EAAC1	0.81	0.56	1.27	2.72	0.80	1.68	1.02	1.28	1.39	1.06	3.81	2.66	0.24	<0.01	<0.01	<0.01	0.35	<0.01	0.77	<0.01
PepT1	1.66	1.24	0.80	1.80	0.96	2.30	0.90	1.22	1.48	1.99	0.83	0.99	0.26	0.97	<0.01	0.24	<0.01	0.34	0.95	<0.01

Values are means plus pooled SEM, n = 8 per treatment group

The mRNA levels for PAT1 and PAT2 (proton-assisted AA transporters), LAT1 (L-type AA transporter 1), ASCT2 (ASC-like Na^+-dependent neutral AA transporter 2), SNAT2 (sodium-coupled neutral amino acid transporter 2), $b^{0,+}$ ($b^{0,+}$ AA transporter), EAAC1 (excitatory AA carrier 1) and PepT1 (H^+/peptide co-transporter) were normalized using GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as an internal control

P phase, *S* strain, *P* × *S* phase × strain interaction, *D* diet, *P* × *D* phase × diet interaction, *S* × *D* strain × diet interaction, *P* × *S* × *D* phase × strain × diet interaction

Table 10 mRNA levels for AA and peptide transporters in *biceps femoris* muscle of growing-finishing pigs

Items	Nursery phase				Growing phase				Finishing phase				SEM	*P* values						
	Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig		Landrace-pig		Bama mini-pig			P_P	P_S	P_{P*S}	P_D	P_{P*D}	P_{S*D}	P_{P*S*D}
	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet	GB diet	NRC diet								
PAT1	0.93	2.45	1.98	2.17	1.36	2.31	3.25	1.64	1.00	0.43	0.62	1.03	0.45	<0.01	0.20	0.78	0.59	0.18	0.09	0.05
PAT2	1.62	1.50	1.52	1.59	1.47	2.27	2.15	1.67	1.52	0.86	0.88	2.79	0.31	0.25	0.26	0.35	0.21	0.40	0.22	<0.01
LAT1	1.36	1.07	1.57	1.85	0.85	0.97	0.70	1.15	0.88	1.15	0.98	1.91	0.17	<0.01	<0.01	0.14	<0.01	0.08	0.02	0.82
ASCT2	0.90	0.75	1.04	1.33	1.07	0.75	0.76	0.85	0.95	1.86	1.67	2.51	0.15	<0.01	<0.01	<0.01	<0.01	<0.01	0.17	0.48
SNAT2	0.61	0.53	1.46	1.82	2.30	2.97	1.30	1.28	1.68	0.45	1.98	1.78	0.31	<0.01	0.37	<0.01	0.68	0.09	0.52	0.21
$b^{0,+}$	1.14	1.13	1.94	1.25	1.24	0.85	2.28	1.36	1.60	3.15	2.75	1.78	0.37	<0.01	0.12	0.33	0.31	0.27	0.01	0.18
EAAC1	0.88	1.93	1.92	0.66	1.71	0.97	1.18	1.82	1.75	1.30	2.01	2.43	0.31	0.08	0.22	0.24	0.78	0.98	0.96	<0.01
PepT1	0.53	0.97	1.08	1.71	0.75	1.20	2.12	2.53	2.09	2.16	2.38	1.05	0.26	<0.01	<0.01	<0.01	0.50	0.01	0.21	0.12

Values are means plus pooled SEM, n = 8 per treatment group

mRNA levels for PAT1 and PAT2 (proton-assisted AA transporters), LAT1 (L-type AA transporter 1), ASCT2 (ASC-like Na^+-dependent neutral AA transporter 2), SNAT2 (sodium-coupled neutral amino acid transporter 2), $b^{0,+}$ ($b^{0,+}$ AA transporter), EAAC1 (excitatory AA carrier 1) and PepT1 (H^+/peptide co-transporter) were normalized using GAPDH (glyceraldehyde-3-phosphate dehydrogenase) as an internal control

P phase, *S* strain, *P* × *S* phase × strain interaction, *D* diet, *P* × *D* phase × diet interaction, *S* × *D* strain × diet interaction, *P* × *S* × *D* phase × strain × diet interaction

deposition (Nishimura and Naito 2008). Accordingly, Bama mini-pigs may have the potential to transport more neutral AA and glutamate, but fewer oligopeptides than Landrace pigs, because PepT1 is a proton-dependent transporter for di- and tri-peptides (Daniel 2004). Further investigation is required to test this hypothesis.

The growth and development of pigs involves not only changes in weight and shape, but also alterations in chemical composition and physiological functions (Blachier et al. 2013; Wu et al. 2004; Hu et al. 2015). In the present study, the mRNA levels of most AA transporters increased with age, such as SNAT2 in LDM, and $b^{0,+}$ AT and PepT1 transporters in BFM. Similarly, Feng et al. (2008) showed that mRNA levels for $b^{0,+}$ AT and y^+ LAT1 transporters in crossbred growing pigs increased with age. In contrast, the mRNA levels for PAT1 in both LDM and BFM increased during the growing phase and decreased during the finishing phase, while the mRNA levels for PAT2 and EAAC1 in LDM and LAT1 and ASCT2 in BFM decreased during the growing phase but increased during the finishing phase. These differences may be due to changes in AA requirements in response to physiological alterations in animals (Hou et al. 2015; Wu et al. 2013). We also found that the mRNA level for the $b^{0,+}$ AT transporter decreased in LDM but increased in BFM with advancing age. The system $b^{0,+}$ is an antiporter that takes up cationic AAs and Cys in exchange for neutral AAs (Chen et al. 2009; Wang et al. 2013). Differences in muscle-subtype may explain the variation in expression of this AA transporter.

Food intake stimulates muscle protein synthesis, which is triggered by the postprandial rise in AAs (Suryawan and Davis 2011). Diets with high levels of AAs may further stimulate protein synthesis, as found in our study in which the NRC diet increased mRNA levels for AA transporters, including PAT1, LAT1, and PepT1 in LDM and of LAT1 and ASCT2 in BFM. Thus, dietary protein affects the growth performance and excretion of dietary nitrogen in pigs (He et al. 2015). According to previous studies (Wu 1998, 2011; Kong et al. 2009), higher levels of nutrients, especially AAs, that enter the portal vein from the small intestine can promote tissue protein synthesis in animals. Therefore, the NRC diet that had a higher level of protein may improve the absorption of dietary AAs and may also directly regulate the metabolism of absorbed nutrients through a signal transduction mechanism. In this regard, it is noteworthy that expression of the proton-assisted AA transporters PAT1 and PAT2 are affected by dietary protein intake. PAT1 and PAT2 are not only responsible for the transport of a variety of small neutral AAs (Goberdhan et al. 2005), but they also have the capability to act as transceptors (Goberdhan 2010) to affect muscle protein metabolism.

In summary, the genetic background and dietary level of protein intake markedly affected free AA concentrations in pig plasma and skeletal muscle, as well as mRNA levels for key AA receptors and transporters in skeletal muscle. These effects of genotype and diet varied with the developmental stage of the animals. Collectively, our findings provide a molecular basis for future development of effective nutritional strategies to increase nutrient utilization in pig production.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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