Original Research

Changes in growth performance, plasma metabolite concentrations, and myogenic gene expression in growing pigs fed a methionine-restricted diet

Zhongyue Yang[1], Md Shamimul Hasan[1], Rebecca M. Humphrey[1], John K. Htoo[2], Shengfa F. Liao[1,*]

[1]*Department of Animal and Dairy Sciences, Mississippi State University, Starkville, MS 39759, USA,* [2]*Evonik Operations GmbH, Rodenbacher Chaussee 4, 63457 Hanau-Wolfgang, Germany*

TABLE OF CONTENTS

1. Abstract

Background: Methionine (Met) is usually the second or third limiting amino acid in swine diets and plays vital roles in promoting the growth, especially, the muscle growth of pigs. This research evaluated the effects of dietary Met restriction on the growth performance, plasma metabolite concentrations, and myogenic gene expression in growing pigs. **Materials and methods**: Eight genes in two families (myogenic regulatory factor family and myocyte enhancer factor 2 family) were selected for the analysis. Twenty individually penned barrows (crossbred, 23.6 $\pm$ 2.4 kg) were randomly allotted to two dietary treatments (n = 10). A diet based on corn and soybean meal (Diet 1, Met-restricted) was formulated to meet or exceed the energy and nutrient requirements, except for Met. Diet 2 (Met-adequate) was formulated by adding crystalline DL-Met to Diet 1 to meet the Met requirement. During the 4-week feeding trial, average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were measured. Immediately before and after the feeding trial, blood was sampled via jugular venipuncture for plasma nutrient metabolite analysis, while *Longissimus dorsi* muscle were sampled via aseptic biopsy for gene expression analysis. Data were analyzed with Student *t*-test. **Results**: Pigs fed the Met-restricted diet had lower ADG and G:F ($P < 0.01$). Plasma Met, cysteine, and taurine concentrations were lower ($P < 0.05$), while glycine and histidine concentrations were higher ($P < 0.05$), in pigs fed the Met-restricted diet. Furthermore, the pigs fed the Met-restricted diet tended to express less myogenic factor 6 (*Myf6*) and myocyte enhancer factor 2D (*Mef2D*) mRNA in *longissimus dorsi* muscle ($P < 0.09$). **Conclusion**: Given the fact that Myf6, assisted by Mef2D, is involved in myocyte

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differentiation, this study suggests that the reduced growth performance in the Met-restricted pigs may be associated with a reduced muscle cell differentiation.

2. Introduction

Known as 2-amino-4-methylthio butanoic acid in chemistry, methionine (Met) is usually the second or third limiting amino acid (AA) in typical swine diets [1]. Commercial product of crystalline DL-Met that consists of 50% D-Met and 50% L-Met is commonly added to the swine diets that are low in Met content [1, 2]. In addition to functioning as a building block for body protein biosynthesis [3, 4], Met has several other biological functions that include (1) protein translation initiation, (2) methyl donation, (3) sulfur source, (4) endogenous antioxidant, (5) precursor of bioactive compounds such as taurine, glutathione, choline, and betaine, and (6) an intermediary in the synthesis of cysteine (Cys) or cystine [1, 5]. Previous research has showed that either a deficiency or a surplus of dietary Met would depress the weight gain and feed efficiency in growing and finishing pigs [6]. The beneficial effects of dietary Met at an optimal level on the growth performance and meat yield of pigs have also been previously reported [5, 7–9]; however, the regulatory molecular mechanisms through which Met regulates the skeletal muscle formation and growth in pigs are still unclear [1, 2].

As is known, myogenesis is a biochemical process of muscle formation regulated by a broad spectrum of cell signaling molecules [10] which are affected by nutrient availability and nutrient metabolism [11]. Among the hierarchical interactions between those molecules and nutrient metabolites, the families of myogenic regulatory factors (MRF) and myocyte enhancer factor 2 (MEF2) for the transcription factor-mediated regulation, are key regulators of muscle growth and differentiation and have been a focus of many previous studies in humans and animals [12, 13]. However, until now little is known about the effects of nutrient Met, a functional AA, on the expressions of these factor genes in pigs.

The MRF family comprise myogenic differentiation 1 (*MyoD or MyoD1*; a.k.a. myoblast determination protein 1), myogenin (*MyoG;* a.k.a. myogenic factor 4, *Myf4*), myogenic factor 5 (*Myf5*), and myogenic factor 6 (*Myf6;* a.k.a. myogenic regulatory factor 4, *Mrf4*), while the MEF2 family comprise *Mef2A*, *Mef2B*, *Mef2C*, and *Mef2D* [10, 14]. Therefore, the objectives of this study were to evaluate (1) the growth performance, (2) the nutrient metabolite profile in the blood, and (3) the expression of these eight genes in skeletal muscle, of young growing pigs when a Met restricted diet was fed.

3. Materials and methods

3.1 Animal trial and growth performance evaluation

The experimental protocol involving caring, handling, and treatment of pigs was approved by the Mississippi State University Institutional Animal Care and Use Committee. Twenty crossbred young growing barrows (Yorkshire × Landrace; initial BW 8.1 ± 0.9 kg) purchased from a local commercial farm were transferred to an environment-controlled swine barn at the Leveck Animal Research Center of Mississippi Agricultural and Forestry Experiment Station. Upon arrival, pigs were randomly assigned to 5 feeding pens and fed a commercial nursery diet until their BW reached 23.6 ± 2.4 kg, during which time the pigs were allowed *ad libitum* access to the diet and fresh water. Pigs were then randomly assigned to 20 individual pens, and further randomly allotted to 2 dietary treatments according to a completely randomized experimental design with pig serving as experimental unit.

A corn and soybean meal based diet (Diet 1, a Met-restricted diet) was formulated to meet or exceed the recommended requirements for energy, crude protein, essential AA, minerals, and vitamins, except for Met [4, 15]. Diet 2 (a Met-adequate diet) was produced by supplementing a commercial product of crystalline DL-Met (99% purity; Evonik Operations GmbH, Hanau-Wolfgang, Germany) to Diet 1 at the expense of corn to meet the requirement of pigs for Met [4, 15]. The diet composition and the calculated nutrient contents are both shown in Table 1 (Ref. [16]), which demonstrates that Diet 2 was a Met-adequate diet while Diet 1 was deficient in SID Met by roughly 40.5%.

To confirm the contents of major nutrients, samples of the two diets were submitted to the Essig Animal Nutrition Laboratory at Mississippi Agricultural and Forestry Experiment Station for proximate and energy analyses, and to the Evonik's chemical laboratory at Hanau-Wolfgang, Germany for AA analysis. For proximate analysis, the contents of dry matter, crude protein, crude fat, crude fiber, and ash were determined according to AOAC International (2000) [17] official methods 9340.01, 2001.11, 920.39, 92.09, and 924.05, respectively. Gross energy was determined using a Parr 1261 Isoperibol Bomb Calorimeter (Parr Instrument Company, Moline, IL, USA). Amino acids were analyzed using ion-exchange chromatography [18, 19]. Tryptophan was analyzed by high-performance liquid chromatography with fluorescence detection [20]. The determined compositions of selected nutrients contained in the diets are shown in Table 2 (Ref. [16]), which indicate that Diet 1 was a diet deficient in total Met by roughly 35.1%.

During the four-week feeding trial, pigs had *ad libitum* access to the experimental diets and fresh water. All feeders, waterers, and pigs were checked at least twice a day (0600 to 2100 hr) to ensure proper function of the facilities and healthy animal behavior. Feed refusals and spillage

Table 1. The ingredient and calculated nutrient compositions of the two experimental diets fed to the young growing pigs (as-fed basis)[1].

Item	Dietary treatment	
	Diet 1	Diet 2
Ingredient, %		
Corn	79.03	78.88
Soybean meal	17.00	17.00
Poultry fat	0.01	0.01
L-lysine HCl, 78.8%	0.62	0.62
DL-methionine, 99%	–	0.15
L-threonine, 98.5%	0.27	0.27
L-tryptophan, 98%	0.09	0.09
L-isoleucine, 96%	0.10	0.10
L-valine, 96.5%	0.18	0.18
L-cysteine HCl, 76.9%	0.11	0.11
Limestone	0.81	0.81
Dicalcium phosphate	1.40	1.40
Salt	0.18	0.18
Mineral premix[2]	0.10	0.10
Vitamin premix[2]	0.10	0.10
Total	100.00	100.00
Major nutrients, %, calculated		
Dry matter	86.48	86.50
Net energy[3], kcal/kg	2,545	2,547
Crude protein	15.42	15.50
SID[4] crude protein	13.10	13.20
SID lysine	1.08	1.08
SID methionine	0.22	0.37
SID methionine + cysteine	0.52	0.67
SID threonine	0.72	0.72
SID tryptophan	0.22	0.22
SID valine	0.76	0.76
SID isoleucine	0.60	0.60
SID leucine	1.20	1.19
Total calcium	0.67	0.67
STTD[5] phosphorus	0.38	0.38
Crude fiber	2.26	2.26
Ash	2.02	2.02

[1]Theses two diets were also used in one of our previously study reported by Humphrey *et al*. [16]. L-lysine HCl and L-threonine were purchased from Archer Daniels Midland Co. (Quincy, IL, USA). L-tryptophan and L-valine were donated from Ajinomoto Heartland, Inc. (Chicago, IL, USA). L-cysteine HCl was purchased from Wuhan Grand Hoyo Co., Ltd. (Wuhan, Hubei, China).

[2]Mineral premix (No. NB-8534) and vitamin premix (No. NB-6508A) were donated from Nutra Blend, LLC. (Neosho, MO, USA). The calculated mineral and vitamin contents in both diets were (per kg of diet): Na, 1.0 g; Cl, 2.9 g; K, 5.6 g; Mg, 1.3 g; S, 1.4 g; Cu, 16.3 mg; Fe, 169.9 mg; I, 0.20 mg; Mn, 37.1 mg; Zn, 131.3 mg, Se, 0.28 mg; vitamin A, 4,401 IU; vitamin D3, 550 IU; vitamin E, 35.6 IU; vitamin K, 1.76 mg; vitamin B1, 2.34 mg; vitamin B2, 4.61 mg; niacin, 42.5 mg; vitamin B5, 16.3 mg; vitamin B6, 5.04 mg; biotin, 0.09 mg; folacin, 0.35 mg; vitamin B12, 15.4 μg, and choline, 1.35 mg.

[3]The unit for energy content was not %, but kcal/kg.

[4]SID, standardized ileal digestible.

[5]STTD, standardized total tract digestible.

Table 2. The analyzed nutrient composition (%, or as indicated) of the two experimental diets fed to the young growing pigs (as-fed basis)[1].

Nutrient and energy[2]	Dietary treatment	
	Diet 1	Diet 2
Proximate analysis		
Dry matter	87.35	87.67
Gross energy, kcal/kg	3,847	3,935
Crude protein	15.00	15.08
Crude fat	1.64	1.95
Crude fiber	1.66	1.76
Ash	4.23	4.42
Amino acid, total		
Lysine	1.17	1.15
Methionine	0.24	0.37
Cysteine	0.34	0.34
Methionine + Cysteine	0.59	0.70
Threonine	0.78	0.77
Tryptophan	0.23	0.23
Arginine	0.91	0.90
Histidine	0.39	0.39
Leucine	1.35	1.36
Isoleucine	0.66	0.65
Valine	0.82	0.83
Phenylalanine	0.72	0.71
Tyrosine	0.37	0.38
Proline	0.94	0.93
Aspartic acid	1.35	1.34
Glutamic acid	2.60	2.59
Serine	0.72	0.71
Alanine	0.82	0.82
Glycine	0.60	0.60
Supplemented free amino acid		
Lysine	0.43	0.45
Methionine	0.00	0.13
Threonine	0.23	0.25
Valine	0.16	0.17

[1]This table was previously reported by Humphrey *et al*. [16] from our research group.

[2]Proximate and energy analyses were conducted at the Essig Animal Nutrition Laboratory, Mississippi Agricultural and Forestry Experiment Station (Starkville, MS, USA). Amino acid analyses were conducted at the analytical laboratories of Ajinomoto Heartland, Inc. (Chicago, IL, USA) and Evonik Operations GmbH (Hanau-Wolfgang, Germany). The amino acid values presented are the mean values from the two laboratory analyses.

were collected and immediately returned to the feeders or reserved and weighed for feed intake calculation. Pigs' BW were measured immediately before, and also at the end of, the four-week feeding trial. The average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were then calculated accordingly.

3.2 Sample collection for laboratory analyses

Immediately before the beginning and at the end of the feeding trial, blood samples (approximately 10 mL/pig) were collected by jugular venipuncture of individual pigs [in a non-fasting state as in an industry setting where pigs are not fasted] in early morning (between 0600 and 0800 hr). The remaining feeds in all the feeders, however, were removed approximately 30 to 60 minutes before the blood collection. Blood samples were kept on ice immediately after the collection until plasma was separated within 30 to 60 minutes through centrifugation at 800 × g and 4 °C for 16 min. Plasma samples in 500-μL aliquots were then stored at –80 °C until laboratory analysis of nutrient metabolites including AA.

After blood collection, a muscle sample (about 200 mg/pig) was collected from the middle portion (the left side) of *longissimus dorsi* muscle of each pig using our standard aseptic biopsy protocol [21]. All muscle samples collected were snap frozen in liquid nitrogen, and then transferred to a –80 °C freezer for storage until gene expression analyses.

3.3 Analyses of plasma nutrient metabolites

The concentrations of plasma free AA were determined at the analytical laboratories of Ajinomoto Heartland, Inc. (Chicago, IL, USA) and Evonik Operations GmbH (Hanau-Wolfgang, Germany) using the official standard high-performance liquid chromatography methods [17]. The principles and procedures of the methods were briefly described by Regmi *et al*. [22] previously.

Batch analysis using the automated ACE Alera Clinical Chemistry System (Alfa Wassermann, West Caldwell, NJ, USA) was performed at the College of Veterinary Medicine Diagnostic Laboratory of Mississippi State University for determination of the concentrations of six representative plasma metabolites with six respective ACE reagents (Alfa Wassermann), and these six metabolites are urea nitrogen, albumin, total protein, glucose, triglycerides, and total cholesterol. The principles and procedures of these laboratory analyses were briefly described by Regmi *et al*. [23] previously.

3.4 Analysis of myogenic gene expression

The myogenic gene expression was analyzed by following our previously reported protocols [24]. Briefly, the total RNA was extracted from approximately 50 mg of muscle sample per pig using TRIzol Reagent (Invitrogen Corporation, Carlsbad, CA, USA) following the manufacturer's instructions. Briefly, a frozen sample was homogenized in a 15-mL polypropylene centrifuge tube using a Polytron mixer (0.5 mL TRIzol per 50 mg tissue), and the homogenate was transferred to a 1.5-mL micro-centrifuge tube. Chloroform (400 μL/tube) was used to separate RNA from DNA and proteins, and then the total RNA was precipitated with isopropyl alcohol (at a ratio of 1:1) and washed with 750 μL of 75% ethanol. The resulted RNA was air-dried, dissolved in 60 μL RNase-free water, and stored in a freezer at –80 °C. The purity and concentration of the total RNA samples were checked by using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

First-strand cDNA was reverse-transcribed from 1 μg of total RNA by using QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA, USA). The semi-quantitative polymerase chain reaction (PCR) analysis was performed using the Rotor-Gene SYBR Green PCR Kit with the Rotor-Gene Q System (Qiagen), followed by melting curve analysis to verify the specificity and identity of the PCR products [24]. The thermal cycling parameters were 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 10 s. Primers for the selected myogenesis-related genes, as well the hypoxanthine phosphoribosyl transferase 1 (*Hprft1*; as the endogenous control) gene, were manufactured at Integrated DNA Technologies (Coralville, IA, USA) with the primer sequences being adopted from Yang *et al*. [24]. The *Hprft1* gene was used for normalization of the potential variations caused during the sample preparation [25].

The comparative $\Delta\Delta C_T$ method was used for mRNA quantity calculation. Briefly, the raw quantity of a given gene was normalized against the raw quantity of *Hprft1* reference gene of a given sample obtained from the Rotor-Gene Q System, and then the normalized level of the given gene of each sample in the Diet 1 group was expressed as a quantity relative to the mean of the normalized quantities of the given gene in the Diet 2 samples [24].

3.5 Statistical analysis

Data were subjected to statistical analysis with Student *t*-test using the SAS software (version 9.4; SAS Institute Inc., Cary, NC) with pigs being the experimental units. A *P*-value less than or equal to 0.05 was considered as having a significant difference between treatment means, and a *P*-value between 0.05 and 0.10 as having a tendency to be different. Each value of the measurements is presented as mean ± standard deviation (SD).

4. Results and discussion

4.1 Growth performance

As shown in Table 3, there was no difference in the initial BW between the two dietary treatment groups ($P > 0.10$). At the end of the feeding trial, even though the final BW of the pigs fed Diet 1 and Diet 2 were not significantly different ($P > 0.10$), the ADG of the pigs fed Diet 1 was significant lower ($P < 0.01$) than that of the pigs fed Diet 2. There was no difference ($P > 0.10$) existed in the ADFI between the two groups. Thus, the G:F of the pigs fed the Met-restricted diet was lower ($P < 0.01$) than that of the pigs fed the Met-adequate diet. These results indicated that a restriction of dietary SID Met by 40.5% did not

affect pig feed intake, but significantly decreased their G:F and ADG. This result of compromised growth performance (i.e., reduced G:F and ADG) is in line with several previous researches, such as those conducted by Bell *et al.* [7], Chung *et al.* [6], Ly *et al.* [9], and Humphrey *et al.* [16], who all reported that dietary Met restriction or deficiency can compromise pig growth performance.

Table 3. The growth performance of the young growing pigs fed with the two experimental diets[1].

Item	Dietary treatment		*P*-value[2]
	Diet 1	Diet 2	
Initial body weight, kg	23.54 ± 2.66	23.64 ± 2.27	0.935
Final body weight, kg	47.44 ± 4.85	50.55 ± 3.37	0.113
Average daily gain, kg	0.87^{a} ± 0.09	0.98^{b} ± 0.06	0.006
Average daily feed intake, kg	1.94 ± 0.24	1.96 ± 0.22	0.849
Gain:feed ratio	0.45^{a} ± 0.02	0.50^{b} ± 0.04	0.001

[1]Diet 1, the methionine-restricted diet; Diet 2, the methionine-adequate diet. Each value is a Mean ± Standard deviation (n = 10).
[2]*P*-values were obtained from Student *t*-test. Means within a row that have different superscripts (a, b) differ ($P < 0.05$ or 0.01).

4.2 Plasma free amino acid profile

In order to further understand how dietary Met restriction could affect nutrient metabolism in growing pigs, the concentrations of plasma free AA were analyzed. As shown in Table 4, prior to the 4-week feeding trial there was no differences ($P > 0.10$) between the two dietary treatment groups in the plasma concentrations of nearly all free AA, except for aspartate. The plasma aspartate concentration tended to be lower in the pigs fed Diet 1 than the pigs fed Diet 2 ($P = 0.07$). As shown in Table 5, after the 4-week feeding trial, the plasma Met concentration was significantly lower in the pigs fed Diet 1 than the pigs fed Diet 2 ($P < 0.01$), and so were the plasma concentrations of Cys and taurine ($P < 0.05$). The plasma concentrations of histidine and glycine, however, were greater ($P < 0.05$) in the pigs fed Diet 1 than fed Diet 2, while the plasma concentrations of lysine and asparagine had tendencies ($0.05 < P < 0.10$) to be greater in the pigs fed Diet 1 than Diet 2. The plasma concentrations of other 17 AAs were not different ($P > 0.11$) between the two dietary treatment groups (Table 5).

The shift of plasma AA profile in pigs fed two different levels of dietary Met is generally in agreement with the results of Li *et al.* [26] and Tian *et al.* [27]. Li *et al.* [26] reported that the plasma concentrations of Met (numerically) and taurine were lower, while the plasma concentrations of lysine (numerically) was higher, in the sows fed a Met adequate vs. a Met excess diet. No data for Cys and glycine from these sows were reported by Li *et al.* [26]. Tian *et al.* [27] reported that the serum concentrations of Met and Cys (numerically) was lower, while the serum concentrations of lysine, histidine (numerically), and glycine were higher, in young growing pigs fed a Met deficient than fed a Met adequate diet. No data for taurine were reported by Tian *et al.* [27].

Table 4. The concentrations of free amino acids in the blood plasma of the young growing pigs before being fed with the two experimental diets[1].

Amino acid, nmol/mL[2]	Dietary treatment		*P*-value[3]
	Diet 1	Diet 2	
Total EAA	1,243 ± 258.9	1,178 ± 332.4	0.659
Methionine	41.4 ± 5.87	38.4 ± 5.50	0.282
Leucine	185.0 ± 33.19	161.3 ± 37.68	0.182
Histidine	118.7 ± 28.61	116.8 ± 35.53	0.903
Phenylalanine	104.4 ± 24.96	99.2 ± 30.47	0.704
Isoleucine	138.2 ± 30.78	125.7 ± 35.08	0.440
Threonine	225.2 ± 91.03	251.5 ± 142.95	0.659
Valine	296.9 ± 59.48	275.0 ± 69.15	0.490
Lysine	65.0 ± 29.41	50.8 ± 13.44	0.192
Tryptophan	67.9 ± 14.13	59.5 ± 22.36	0.366
Total NEAA	3,678 ± 724.9	3,664 ± 899.8	0.972
Arginine	174.4 ± 35.45	144.2 ± 40.16	0.115
Citrulline	94.7 ± 23.35	83.7 ± 20.60	0.304
Alanine	644.6 ± 149.70	613.3 ± 146.70	0.661
Glutamate	270.9 ± 56.59	357.9 ± 186.49	0.190
Glycine	908.8 ± 220.80	823.7 ± 166.50	0.365
Asparagine	112.8 ± 32.41	107.7 ± 35.60	0.756
Aspartate	22.2 ± 5.25	30.7 ± 21.70	0.074
β-Alanine	15.5 ± 1.88	18.8 ± 8.29	0.244
Glutamine	750.5 ± 288.40	844.2 ± 411.59	0.594
Ornithine	178.0 ± 39.89	156.4 ± 35.61	0.242
Serine	180.3 ± 50.34	160.3 ± 34.58	0.333
Taurine	213.2 ± 99.12	216.8 ± 108.10	0.943
Tyrosine	112.6 ± 36.05	106.5 ± 41.07	0.745
Cysteine	188.6 ± 29.07	186.0 ± 17.99	0.813
Proline	337.2 ± 75.80	350.0 ± 66.27	0.692
Total AA	4,921 ± 944.6	4,843 ± 1,208.1	0.882

[1]The calculated dietary standardized ileal digestible methionine contents (as-fed basis) in Diets 1 and 2 were 0.22% and 0.37%, respectively.
[2]EAA, essential amino acids; NEAA, non-essential amino acids; and total AA include total EAA and total NEAA. Each value is a Mean ± Standard deviation (n = 10).
[3]*P*-values were obtained from Student *t*-test.

Obviously, the lower concentrations of plasma Met in pigs fed diets deficient or low in Met can be attributed to the limited dietary supply of Met in these studies. Given the fact that Cys and taurine are products of Met metabolism [28], the lower Cys and taurine concentrations in the plasma of pigs fed a Met restricted diet might be due to the limited dietary Met supply as well. The higher plasma concentrations of lysine, histidine, and glycine associated with the low dietary Met supply might be attributed to the fact that lysine, histidine, glycine, asparagine, and Met share the $B^{0,+}$ AA transport system in small intestine [26, 29]. Lysine, histidine, asparagine, and Met also share

Table 5. The concentrations of free amino acids in the blood plasma of the young growing pigs after being fed with the two experimental diets for four weeks[1].

Amino acid, nmol/mL[2]	Dietary treatment		*P*-value[3]
	Diet 1	Diet 2	
Total EAA	2,258 ± 1,082.0	1,697 ± 427.1	0.146
Methionine	28.7^{a} ± 6.83	53.6^{b} ± 14.54	<0.001
Leucine	262.1 ± 72.24	224.6 ± 72.56	0.261
Histidine	119.5^{b} ± 41.41	84.1^{a} ± 25.02	0.033
Phenylalanine	88.7 ± 28.89	71.4 ± 15.92	0.115
Isoleucine	162.4 ± 66.94	130.1 ± 39.40	0.205
Threonine	574.7 ± 419.77	372.5 ± 90.68	0.154
Valine	422.5 ± 186.10	358.6 ± 121.56	0.376
Lysine	459.1 ± 250.55	292.6 ± 102.21	0.067
Tryptophan	140.4 ± 59.17	111.3 ± 28.40	0.178
Total NEAA	4,144 ± 1,133.0	3,659 ± 844.3	0.293
Arginine	169.4 ± 63.48	146.8 ± 61.29	0.427
Citrulline	75.8 ± 26.36	63.5 ± 14.30	0.211
Alanine	670.8 ± 228.77	572.2 ± 183.13	0.301
Glutamate	198.8 ± 60.97	256.4 ± 102.82	0.145
Glycine	1162.7^{b} ± 277.29	921.5^{a} ± 171.68	0.031
Asparagine	158.1 ± 97.82	99.3 ± 29.61	0.086
Aspartate	22.3 ± 8.18	21.9 ± 7.77	0.914
β-Alanine	24.2 ± 6.71	26.7 ± 8.24	0.474
Glutamine	936.5 ± 371.69	898.4 ± 257.54	0.793
Ornithine	182.7 ± 46.25	172.9 ± 39.76	0.619
Serine	271.9 ± 122.57	206.8 ± 56.47	0.145
Taurine	113.4^{a} ± 21.67	142.1^{b} ± 37.23	0.049
Tyrosine	157.0 ± 67.06	130.6 ± 32.27	0.276
Cysteine	157.4^{a} ± 16.93	226.4^{b} ± 37.51	<0.001
Proline	327.8 ± 48.55	346.1 ± 52.14	0.427
Total AA	6,402 ± 2,147.1	5,358 ± 1,208.5	0.197

[1]The calculated dietary standardized ileal digestible methionine contents (as-fed basis) in Diets 1 and 2 were 0.22% and 0.37%, respectively.
[2]EAA, essential amino acids, NEAA, non-essential amino acids, and total AA include total EAA and total NEAA. Each value is a Mean ± Standard deviation (n = 10).
[3]*P*-values were obtained from Student *t*-test. Means within a row that have different superscripts (a, b) differ ($P < 0.05$ or 0.01).

the $b^{0,+}$ transport system in small intestine [26, 29]. In addition, the reduced ADG (Table 3) due to the limited supply of Met might also be a reason for the higher plasma concentrations of lysine, histidine, and glycine, because the body protein biosynthesis may be limited and these three AA became extra in the Met restricted pigs.

A high plasma glycine concentration indicates that it was possible that less glycine was utilized for glutathione synthesis due to insufficient Met supply in the diet. Given the fact that both glutathione and taurine (derived from Cys) are essential antioxidants in pig body [1], the reduced plasma concentrations of Met, taurine, and possibly glutathione, might also be responsible for the reduced growth performance of pigs fed the Met-restricted diet. Although the parameters related to oxidative status were not measured in this study, the change in plasma AA concentration indicates that dietary Met restriction may negatively affect pig's antioxidant capacity, health status, and, in consequence, growth performance.

4.3 Plasma concentrations of nutrient metabolites

In addition to the free AA, the plasma concentrations of six major metabolites were also analyzed. As shown in Table 6, before the feeding trial, there were no differences ($P > 0.10$) between the two dietary treatment groups in the plasma concentrations of any one of the metabolites. After the feeding trial, however, the plasma urea nitrogen concentration was greater ($P < 0.01$) in pigs fed Diet 1 than pigs fed Diet 2, while the plasma albumin concentration tended to be greater ($P < 0.09$) in pigs fed Diet 1 than Diet 2. The plasma concentrations of other metabolites, including total protein, total cholesterol, glucose, and triglycerides, were not different ($P > 0.10$) between the two treatment groups. The result of high plasma urea nitrogen concentration associated with the Met-restricted diet obtained in this study is in consistent with the results of some previous studies conducted by Shen *et al.* [5] and Tian *et al.* [27], who reported increased concentrations of plasma urea nitrogen with decreased dietary Met content in nursery and starter pigs, respectively.

Table 6. The concentrations of selected metabolites in the blood plasma of the young growing pigs before and after the four-week feeding trial[1].

Metabolite	Dietary treatment		*P*-value[2]
	Diet 1	Diet 2	
Before the feeding trial			
Urea nitrogen, mg/dL	12.4 ± 2.32	11.1 ± 1.52	0.156
Total protein, g/dL	4.75 ± 0.31	4.59 ± 0.29	0.251
Albumin, g/dL	2.40 ± 0.24	2.34 ± 0.25	0.591
Glucose, mg/dL	111.7 ± 10.8	106.2 ± 9.9	0.251
Total cholesterol, mg/dL	95.4 ± 12.3	87.0 ± 16.1	0.208
Triglycerides, mg/dL	45.2 ± 15.0	47.2 ± 13.2	0.756
After the feeding trial			
Urea nitrogen, mg/dL	6.6^{b} ± 1.17	4.2^{a} ± 1.23	<0.001
Total protein, g/dL	5.71 ± 0.22	5.51 ± 0.44	0.211
Albumin, g/dL	3.64 ± 0.17	3.41 ± 0.36	0.085
Glucose, mg/dL	108.8 ± 12.7	115.0 ± 10.6	0.250
Total cholesterol, mg/dL	77.6 ± 11.2	84.4 ± 10.6	0.180
Triglycerides, mg/dL	40.7 ± 9.5	53.4 ± 22.9	0.123

[1]The calculated dietary standardized ileal digestible methionine contents in Diets 1 and 2 were 0.22% and 0.37% (as-fed basis), respectively. Each value is a Mean ± Standard deviation (n = 10).
[2]*P*-values were obtained from Student *t*-test. Means within a row that have different superscripts (a, b) differ ($P < 0.05$ or 0.01).

The plasma urea nitrogen concentration is a reliable indicator of AA utilization efficiency by the animal [30–32]. The ideal amounts and ratios of all proteinogenic AA are essential for efficient nitrogen utilization, minimal

Table 7. The mRNA levels of myogenic gene expression in the pigs fed a methionine-restricted or -adequate diet before and after the four-week feeding trial[1].

Gene name[2]	Gene symbol	Dietary treatment		*P*-value[3]
		Diet 1	Diet 2	
Before the feeding trial				
Myogenic differentiation 1	*MyoD*	1.34 ± 0.34	1.17 ± 0.59	0.468
Myogenin	*MyoG*	0.95 ± 0.25	1.13 ± 0.61	0.446
Myogenic factor 5	*Myf5*	1.21 ± 0.12	1.04 ± 0.33	0.184
Myogenic factor 6	*Myf6*	1.56 ± 1.42	1.23 ± 0.59	0.524
Myocyte enhancer factor 2A	*Mef2A*	1.40 ± 0.17	1.24 ± 0.26	0.170
Myocyte enhancer factor 2B	*Mef2B*	2.19 ± 2.13	2.25 ± 2.80	0.961
Myocyte enhancer factor 2C	*Mef2C*	1.24 ± 0.20	1.38 ± 0.96	0.687
Myocyte enhancer factor 2D, transcript variant X1	*Mef2D*	0.87 ± 0.48	1.08 ± 0.50	0.383
After the feeding trial				
Myogenic differentiation 1	*MyoD*	1.03 ± 0.50	1.24 ± 0.84	0.521
Myogenin	*MyoG*	1.16 ± 0.63	1.11 ± 0.51	0.855
Myogenic factor 5	*Myf5*	1.50 ± 1.88	1.12 ± 0.76	0.572
Myogenic factor 6	*Myf6*	0.76 ± 0.32	1.10 ± 0.45	0.079
Myocyte enhancer factor 2A	*Mef2A*	1.14 ± 0.65	1.07 ± 0.40	0.796
Myocyte enhancer factor 2B	*Mef2B*	1.17 ± 0.52	1.39 ± 1.14	0.590
Myocyte enhancer factor 2C	*Mef2C*	0.83 ± 0.40	1.16 ± 0.62	0.194
Myocyte enhancer factor 2D, transcript variant X1	*Mef2D*	0.69 ± 0.39	1.13 ± 0.61	0.083

[1] Diet 1, a methionine-restricted diet; Diet 2, a methionine-adequate diet. The calculated dietary standardized ileal digestible methionine contents (as-fed basis) in Diets 1 and 2 were 0.22% and 0.37%, respectively.

[2] Myogenin (*MyoG*) is also known as myogenic factor 4 (*Myf4*). Myogenic factor 6 (*Myf6*) is also known as muscle regulatory factor 4 (*Mrf4*) or herculin.

[3] *P*-values were obtained from Student *t*-test.

AA catabolism, maximal protein synthesis, and thus the least plasma urea nitrogen concentration [11, 23, 33]. Data from this study showed that the plasma urea nitrogen concentration was greater in the Met-restricted pigs than in the Met-adequate pigs. The probable reason for this finding is that in the Met-restricted group, Met was the first limiting AA, and after the Met was exhausted, all other AA became "extra" and were catabolized to produce more urea nitrogen [30].

The plasma albumin concentration is also a good indicator of the effectiveness of dietary AA utilization, and of the liver capacity of protein synthesis [23, 34, 35]. The present study is in consistent with the result of Tian *et al*. [27] for young growing pigs. Our result is also supported by Litvak *et al*. [36] who reported that a low-level Met diet increased serum albumin concentration in growing pigs. It may also be speculated that increased hepatic albumin synthesis reflects increased need for endogenous Met to support the immune response since albumin is used to transfer Met [36].

There was no difference in the plasma total protein concentrations between the pigs fed Diet 1 and Diet 2 ($P > 0.10$), indicating that the dietary Met restriction did not affect the plasma total protein content. This result is consistent with the findings of Chattopadhyay *et al*. [37], Meng *et al*. [38], and Remus *et al*. [39], who reported that the plasma total protein concentrations were not different in animals with different content of Met supply. The unchanged plasma total protein concentration but decreased BW gain may indicate a homeostatic control of plasma total protein concentration and a non-preferential utilization of AA for muscle protein syntheses.

There was no significant difference ($P > 0.10$) between the two treatment groups in the plasma concentrations of glucose, triglycerides, and total cholesterol at the end of the feeding trial. This finding is in agreement with the results of Saeid *et al*. [40], who also found in broilers that Met alone has no regulating effect on plasma concentrations of glucose, triglycerides and cholesterol. The results of the current study and that of Saeid *et al*. [40] may indicate that the restriction of dietary Met had no effects on energy and lipid metabolism.

4.4 Myogenic gene expression

Before the feeding trial, the mRNA levels of the selected myogenic genes were similar ($P > 0.10$) between the Diet 1 and Diet 2 pigs (Table 7). After the four-week feeding trial, however, the pigs fed with Diet 1 tended to have lower levels of *Myf6* ($P < 0.08$) and *Mef2D* ($P < 0.09$) mRNA. There were no differences in the mRNA levels of other 6 myogenic genes between the two groups of pigs ($P > 0.10$). These results indicate that dietary Met restriction may reduce the expression of *Myf6* and *Mef2D* genes, which might reduce the expression levels of the correspond-

ing proteins in the skeletal muscle of growing pigs.

As it is known, the MRF family are highly conserved and collectively expressed in skeletal muscle lineages [10, 14]. Assisted by the MEF2 family, MRFs coordinate the activities of a host of co-activators and co-repressors, resulting in a tight control of gene expression during myogenesis [41, 42]. Either MyoD or Myf5 is sufficient for skeletal muscle formation [43], but MyoG and Myf6 are directly involved in myotube differentiation [10, 44]. Therefore, the present data imply that Met may affect the myotube or muscle cell differentiation, but not the muscle cell formation. The functions of different MEF2 isoforms (activating the muscle structural genes) are difficult to distinguish because they are expressed in distinct but overlapping patterns [2, 45]. The reason why only *Mef2D*, but not the other isoforms, was affected by Met in this study are unknown.

A study conducted in pigs by Li *et al*. [2] showed that dietary Met supplementation increased the mRNA expression levels of *MyoG*, *Mef2A*, and *Mef2D*, but not of *Myf6* (as in this study). The discrepancy between the results of Li *et al*. [2] and of this study might be mainly due to the difference in the animal models used. The low birth weight piglets were used by Li *et al*. [2] without reporting breed and sex, and there was no difference in the growth performance between the control and the Met supplemented pigs [2]. In this study, the muscle samples were collected when pigs were around 80 d of age, while Li *et al*. [2] collected their muscle samples when pigs were 180 d of age.

5. Conclusions

Dietary Met restriction reduced the plasma concentrations of Met, Cys and taurine, but increased or tended to increase the concentrations of histidine, glycine, lysine, and asparagine in growing pigs. The Met restriction also increased or tended to increase the plasma concentration of urea nitrogen and albumin. These results confirmed that insufficient amount of dietary Met as a protein building block was the primary reason for the compromised G:F and ADG of the pigs fed with Met restricted diets, and the compromised G:F and increased plasma concentration of urea nitrogen can be attributed to the reduced efficiency of AA utilization and body protein synthesis. The Met restriction tended to reduce the abundance of *Myf6* and *Mef2D* mRNA in the *longissimus* muscle of the pigs, which suggests that the reduced efficiency of AA utilization and protein synthesis may be associated with a reduced level of myotube differentiation rather than muscle cell formation.

6. Author contributions

SFL and JKH conceived and designed the experiment; ZY, MSH, and RMH performed the experiment; ZY analyzed the data and prepared the first draft of the manuscript; SFL supervised the experiment and finalized the manuscript. All authors have read and approved the final manuscript.

7. Ethics approval and consent to participate

The experimental protocol involving the caring, handling, and treatment of pigs for the experiment was approved by the Mississippi State University Institutional Animal Care and Use Committee (IACUC #16-016).

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10. Conflict of interest

JKH is an employee at Evonik Operations GmbH (Hanau-Wolfgang, Germany), a commercial supplier of DL-methionine to the global feed industry. All other authors have no conflicts of interest regarding the publication of this article.

11. References

[1] Yang Z, Htoo JK, Liao SF. Methionine nutrition in swine and related monogastric animals: Beyond protein biosynthesis. Animal Feed Science and Technology. 2020; 268: 114608.

[2] Li Y, Zhang H, Chen YP, Ying ZX, Su WP, Zhang LL, *et al*. Effects of dietary l-methionine supplementation on the growth performance, carcass characteristics, meat quality, and muscular antioxidant capacity and myogenic gene expression in low birth weight pigs. Journal of Animal Science. 2017; 95: 3972–3983.

[3] Lewis AJ. Amino acids in swine nutrition. In Lewis, A.J., Southern, L.L. (eds.) Swine Nutrition (pp. 131–150). 2nd edn. CRC Press: Boca Raton. 2001.

[4] National Research Council. Nutrient Requirements of Swine. 11th edn. National Academies Press: Washington, D.C. 2012.

[5] Shen YB, Weaver AC, Kim SW. Effect of feed grade L-methionine on growth performance and gut health in nursery

pigs compared with conventional DL-methionine. Journal of Animal Science. 2014; 92: 5530–5539.
[6] Chung TK, Izquierdo OA, Hashimoto K, Baker DH. Methionine requirement of the finishing pig. Journal of Animal Science. 1989; 67: 2677–2683.
[7] Bell JM, Williams HH, Loosli JK, Maynard LA. The Effect of Methionine Supplementation of a Soybean Oil Meal—Purified Ration for Growing Pigs: Two figures. The Journal of Nutrition. 1950; 40: 551–561.
[8] Owen KQ, Goodband RD, Nelssen JL, Tokach MD, Dritz SS. The effect of dietary methionine and its relationship to lysine on growth performance of the segregated early-weaned pig. Journal of Animal Science. 1995; 73: 3666–3672.
[9] Ly NTH, Ngoan LD, Verstegen MWA, Hendriks WH. Pig performance increases with the addition of DL-methionine and L-lysine to ensiled cassava leaf protein diets. Tropical Animal Health and Production. 2012; 44: 165–172.
[10] Bentzinger CF, Wang YX, Rudnicki MA. Building muscle: molecular regulation of myogenesis. Cold Spring Harbor Perspectives in Biology. 2012; 4: a008342.
[11] Liao SF, Wang T, Regmi N. Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond. SpringerPlus. 2015; 4: 147.
[12] Townley-Tilson WHD, Callis TE, Wang D. MicroRNAs 1, 133, and 206: critical factors of skeletal and cardiac muscle development, function, and disease. The International Journal of Biochemistry & Cell Biology. 2010; 42: 1252–1255.
[13] Schiaffino S, Dyar KA, Calabria E. Skeletal muscle mass is controlled by the MRF4-MEF2 axis. Current Opinion in Clinical Nutrition and Metabolic Care. 2018; 21: 164–167.
[14] Zammit PS. Function of the myogenic regulatory factors Myf5, MyoD, Myogenin and MRF4 in skeletal muscle, satellite cells and regenerative myogenesis. Seminars in Cell & Developmental Biology. 2017; 72: 19–32.
[15] Evonik Industries. AMINODat. (5.0; Platinum version). Evonik Nutrition & Care GmbH: Hanau-Wolfgang, Germany. 2016.
[16] Humphrey RM, Yang Z, Hasan MS, Crenshaw MA, Burnett DD, Htoo JK, *et al*. The compensatorily-gained pigs resulted from feeding a methionine-deficient diet had more fat and less lean body mass. Journal of Applied Animal Nutrition. 2018; 6: e6.
[17] AOAC International. Official methods of analysis of AOAC (Association of Official Analytical Chemists) international. 17th edn. AOAC International: Gaithersburg, MD, USA. 2000.
[18] Llames CR, Fontaine J. Determination of Amino Acids in Feeds: Collaborative Study. Journal of AOAC International. 1994; 77: 1362–1402.
[19] Directive C. Establishing community methods for the determination of amino acids, crude oils and fats, and olaquindox in feeding stuff and amending directive 71/393/EEC, annex part a. Determination of amino acids. Official Journal of the European Communities. 1998; 257: 14–23.
[20] Directive C. Establishing community methods of analysis for the determination of vitamin A, vitamin E and tryptophan in feedstuffs: Annex part C: determination of tryptophan. Official Journal of the European Communities. 2000; 174: 45–50.
[21] Burnett DD, Paulk CB, Tokach MD, Nelssen JL, Vaughn MA, Phelps KJ, *et al*. Effects of Added Zinc on Skeletal Muscle Morphometrics and Gene Expression of Finishing Pigs Fed Ractopamine-HCL. Animal Biotechnology. 2015; 27: 17–29.
[22] Regmi N, Wang T, Crenshaw MA, Rude BJ, Wu G, Liao SF. Effects of dietary lysine levels on plasma free amino acid profile in late-stage finishing pigs. SpringerPlus. 2016; 5: 888.
[23] Regmi N, Wang T, Crenshaw MA, Rude BJ, Liao SF. Effects of dietary lysine levels on the concentrations of selected nutrient metabolites in blood plasma of late-stage finishing pigs. Journal of Animal Physiology and Animal Nutrition. 2018; 102: 403–409.
[24] Yang Z, Hasan MS, Htoo JK, Burnett DD, Feugang JM, Crenshaw MA, *et al*. Effects of dietary supplementation of l-methionine vs. dl-methionine on performance, plasma concentrations of free amino acids and other metabolites, and myogenesis gene expression in young growing pigs. Translational Animal Science. 2018; 3: 329–339.
[25] Nygard A, Jørgensen CB, Cirera S, Fredholm M. Selection of reference genes for gene expression studies in pig tissues using SYBR green qPCR. BMC Molecular Biology. 2007; 8: 67.
[26] Li H, Wan H, Mercier Y, Zhang X, Wu C, Wu X, *et al*. Changes in plasma amino acid profiles, growth performance and intestinal antioxidant capacity of piglets following increased consumption of methionine as its hydroxy analogue. British Journal of Nutrition. 2014; 112: 855–867.
[27] Tian QY, Zeng ZK, Zhang YX, Long SF, Piao XS. Effect of L- or DL-methionine Supplementation on Nitrogen Retention, Serum Amino Acid Concentrations and Blood Metabolites Profile in Starter Pigs. Asian-Australasian Journal of Animal Sciences. 2016; 29: 689–694.
[28] Wu G. Amino acids: biochemistry and nutrition. 1st edn. CRC Press: Boca Raton. 2013.
[29] Bröer S. Amino acid transport across mammalian intestinal and renal epithelia. Physiological Reviews. 2008; 88: 249–286.
[30] Coma J, Zimmerman DR, Carrion D. Relationship of rate of lean tissue growth and other factors to concentration of urea in plasma of pigs. Journal of Animal Science. 1995; 73: 3649–3656.
[31] Chen HY, Lewis AJ, Miller PS, Yen JT. The effect of excess protein on growth performance and protein metabolism of finishing barrows and gilts. Journal of Animal Science. 1999; 77: 3238–3247.
[32] Chen HY, Lewis AJ, Miller PS, Yen JT. The effect of infusion of urea into the vena cava on feed intake of finishing gilts. Journal of Animal Science. 1999; 77: 3248–3252.
[33] Brown JA, Cline TR. Urea excretion in the pig: an indicator of protein quality and amino acid requirements. The Journal of Nutrition. 1974; 104: 542–545.
[34] Lowrey RS, Pond WG, Barnes RH, Krook L, Loosli JK. Influence of caloric level and protein quality on the manifestations of protein deficiency in the young pig. Journal of Nutrition. 1962; 78: 245–253.
[35] Mahdavi A, Shivazad M, Alemi F, Zaghari M, Moravej H, Darabighane B. Digestible lysine requirement of broilers based on practical diet. Italian Journal of Animal Science. 2012; 11: e13.
[36] Litvak N, Rakhshandeh A, Htoo JK, de Lange CFM. Immune system stimulation increases the optimal dietary methionine to methionine plus cysteine ratio in growing pigs. Journal of Animal Science. 2013; 91: 4188–4196.
[37] Chattopadhyay K, Mondal MK, Roy B. Comparative efficacy of DL-methionine and herbal methionine on performance of broiler chicken. International Journal of Poultry Science. 2006; 5: 1034–1039.
[38] Meng GH, Song D, Li LB, Yang CJ, Qu ZX, Gao YP. Dietary methionine requirement of Jing Brown layer hens from 9 to 17 weeks of age. Journal of Animal Physiology and Animal Nutrition. 2017; 101: 925–935.
[39] Remus A, Pomar C, Perondi D, Gobi JP, da Silva WC, de Souza LJ, *et al*. Response to dietary methionine supply of growing pigs fed daily tailored diets or fed according to a conventional phase feeding system. Livestock Science. 2019; 222: 7–13.
[40] Saeid SS, Reza YM, Javad N. The effects of different levels of L-carnitine and methionine on performance and blood metabolites in female broiler. Research Opinions in Animal and Veterinary Sciences. 2014; 4: 427–431.
[41] Black BL, Olson EN. Transcriptional control of muscle development by myocyte enhancer factor-2 (MEF2) proteins. Annual Review of Cell and Developmental Biology. 1998; 14: 167–196.
[42] Hernández-Hernández JM, García-González EG, Brun CE, Rudnicki MA. The myogenic regulatory factors, determinants of muscle development, cell identity and regeneration. Seminars in Cell & Developmental Biology. 2017; 72: 10–18.

[43] Rudnicki MA, Schnegelsberg PN, Stead RH, Braun T, Arnold HH, Jaenisch R. MyoD or Myf-5 is required for the formation of skeletal muscle. Cell. 1993; 75: 1351–1359.
[44] Brameld JM, Buttery PJ, Dawson JM, Harper JM. Nutritional and hormonal control of skeletal-muscle cell growth and differentiation. The Proceedings of the Nutrition Society. 1998; 57: 207–217.
[45] McKinsey TA, Zhang CL, Olson EN. MEF2: a calcium-dependent regulator of cell division, differentiation and death. Trends in Biochemical Sciences. 2002; 27: 40–47.

Abbreviations: Met, methionine; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain, feed ratio; AA, amino acids; Cys, cysteine; MRF, myogenic regulatory factors; MEF2, myocyte enhancer factor 2; MyoD or MyoD1, myogenic differentiation 1; MyoG, myogenin; Myf5, myogenic factor 5; Myf6, myogenic factor 6.

Keywords: Methionine; Myogenic gene; Plasma metabolite; Amino acid; Pig

Send correspondence to: Shengfa F. Liao, Department of Animal and Dairy Sciences, Mississippi State University, Starkville, MS 39759, USA, E-mail: s.liao@msstate.edu