NON RUMINANT NUTRITION

Dietary lysine affects amino acid metabolism and growth performance, which may not involve the GH/IGF-1 axis, in young growing pigs¹

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Abstract

Lysine is the first limiting amino acid (AA) in typical swine diets. Our previous research showed that dietary lysine restriction compromised the growth performance of late-stage finishing pigs, which was associated with the changes in plasma concentrations of nutrient metabolites and hormone insulin-like growth factor 1 (IGF-1). This study was conducted to investigate how dietary lysine restriction affects the plasma concentrations of selected metabolites and three anabolic hormones in growing pigs. Twelve individually penned young barrows (Yorkshire × Landrace; 22.6 ± 2.04 kg) were randomly assigned to two dietary treatments ($n = 6$). Two corn and soybean meal based diets were formulated to contain 0.65% and 0.98% standardized ileal digestible lysine as a lysine-deficient (LDD) and a lysine-adequate (LAD) diets, respectively. During the 8-week feeding trial, pigs had ad libitum access to water and their respective diets, and the growth performance parameters including average daily gain (ADG), average daily feed intake (ADFI), and gain-to-feed ratio (G:F) were determined. At the end of the trial, jugular vein blood was collected for plasma preparation. The plasma concentrations of free AA and six metabolites were analyzed with the established chemical methods, and the hormone concentrations were analyzed with the commercial ELISA kits. Data were analyzed with Student's *t*-test. The ADG of LDD pigs was lower ($P < 0.01$) than that of LAD pigs, and so was the G:F ($P < 0.05$) since there was no difference in the ADFI between the two groups of pigs. In terms of free AA, the plasma concentrations of lysine, methionine, leucine, and tyrosine were lower ($P < 0.05$), while that of β -alanine was higher ($P < 0.01$), in the LDD pigs. The total plasma protein concentration was lower ($P < 0.02$) in the LDD pigs, whereas no differences were observed for the other metabolites between the two groups. No differences were observed in the plasma concentrations of growth hormone (GH), insulin, and IGF-1 between the two groups as well. These results indicate that the lack of lysine as a protein building block must be the primary reason for a reduced body protein synthesis and, consequently, the compromised G:F ratio and ADG. The changes in the plasma concentrations of total protein and four AA suggest that the compromised growth performance might be associated with some cell signaling and metabolic pathways that may not involve the GH/IGF-1 axis.

Key words: amino acid, growing pig, hormone, lysine, plasma metabolite

Introduction

A primary goal of swine production is to grow skeletal muscle, the major component of pork, for human consumption. It is well known that muscle growth essentially requires dietary supply of proteinogenic amino acids (AA). There are 20 different proteinogenic AA in typical swine diets, and the main function of these AA is to serve as building blocks for syntheses of body proteins, such as muscle proteins (Hou et al., 2015; Regmi et al., 2016). Muscle proteins by nature undergo a continuous turnover that is the old, damaged, or unneeded proteins are degraded, and new proteins are de novo synthesized (Wu et al., 2014; Liao et al., 2015). Therefore, a constant supply of sufficient AA to muscle cells from the blood is required to ensure muscle protein accretion, that is, the rate of protein synthesis is greater than that of protein degradation.

Each protein molecule is a polymer of AA residues joined together by peptide bonds, and each polymer has a unique linear AA sequence and, thus, fixed ratios of different AA. To maximize protein synthesis rate in skeletal muscle, various free AA must be available simultaneously in certain ratios that match muscle protein AA composition (Christensen, 1964; Liao et al., 2015; van Milgen and Dourmad, 2015). Lysine is the first limiting AA in common grain-based swine diets (Liao et al., 2015). Previous research showed that the level of dietary lysine can have significant effect on the growth performance of pigs (O'Connell et al., 2006; Kim et al., 2011; Taylor et al., 2015). Our previous studies (Regmi et al., 2016, 2018; Wang et al., 2017) also showed that dietary lysine deficiency (i.e., restriction) significantly compromised the growth performance of the late-stage finishing pigs, and this compromise was associated with the observed changes in the plasma concentrations of some nutrient metabolites (including some AA) and a growth-related hormone, insulin-like growth factor 1 (IGF-1). However, similar studies on young growing pigs are still limited.

To further understand the regulatory role of dietary lysine on swine growth performance, this study was conducted in young growing pigs with three main objectives, which were to study the effects of dietary lysine restriction on 1) the plasma free AA profile; 2) the plasma concentrations of the selected metabolites related to protein, lipid, and energy metabolism; and 3) the plasma concentrations of three growth-related hormones.

Materials and Methods

Animal and Dietary Treatment

Twelve crossbred (Yorkshire × Landrace) barrows (initial BW 22.6 ± 2.04 kg) in 12 individual pens (24 ft² each) housed in an environment-controlled barn at a Mississippi State University farm were assigned to two dietary treatments according to a completely randomized experimental design with pig serving as experimental unit. A corn and soybean meal-based diet (a lysine-deficient diet, LDD) was formulated (Table 1) to meet or exceed NRC (2012) recommended requirements for various nutrients including crude protein and essential AA, but not lysine. As given in Table 1, the control diet (i.e., a lysine-adequate diet, LAD) was formulated by adding crystalline L-lysine monohydrochloride to the LDD at 0.40% (as-fed basis).

During the course of the feeding trial, diet samples were collected and submitted to the Essig Animal Nutrition Laboratory at Mississippi State University for proximate analysis to confirm the contents of major nutrients. Also, the AA composition of the diets was analyzed in Dr. Guoyao Wu's laboratory at Texas

Table 1. Composition of the two experimental diets fed to the growing pigs (as-fed basis)

Item	Diet*	
	LDD	LAD
<i>Ingredients, %</i>		
Corn	74.752	74.352
Soybean meal	20.000	20.000
Canola oil	2.400	2.400
L-Lysine-HCl†	0.000	0.400
DL-Methionine‡	0.080	0.080
L-Threonine	0.100	0.100
L-Tryptophan§	0.028	0.028
Limestone	0.810	0.810
Dicalcium phosphate	1.400	1.400
Salt	0.180	0.180
Grow/Finish Premix¶	0.250	0.250
<i>Major nutrients, calculated</i>		
Dry matter, %	82.2	82.2
Net energy, kcal/kg	2,382	2,386
SID* crude protein, %	13.2	13.7
SID lysine, %	0.65	0.96
SID methionine, %	0.30	0.30
SID methionine + SID cysteine, %	0.52	0.52
Total calcium, %	0.68	0.68
STTD phosphorus, %	0.31	0.31
Crude fiber, %	2.05	2.04
Ash, %	2.23	2.23

*LDD, the lysine-deficient diet; LAD, the lysine-adequate diet. The calculated total lysine contents (as-fed basis) in LDD and LAD were 0.81% and 1.12%, respectively.

†L-Lysine-HCl (98.5%) and L-threonine (98.5%) were donated by Ajinomoto Heartland, Inc. (Chicago, IL).

‡DL-Methionine (99.0%; Rhodimet, NP 99) was donated by Adisseo USA, Inc. (Alpharetta, GA).

§L-Tryptophan (99.0%) was donated by Ajinomoto Heartland, Inc. (Chicago, IL).

¶Grow/Finish Premix 5 (G07390N) was donated from Archer Daniels Midland Alliance Nutrition (Quincy, IL). The calculated mineral and vitamin contents in both diets were (per kg of diet): S, 0.08 g; Cu, 13.5 mg; Fe, 123.8 mg; I, 0.28 mg; Mn, 27.0 mg; Zn, 123.8 mg; Se, 0.30 mg; vitamin A, 4,953 IU; vitamin D₃, 594 IU; vitamin E, 26.4 IU; vitamin K, 2.18 mg; vitamin B₁₂, 4.95 mg; niacin, 24.8 mg; vitamin B₅, 19.8 mg; and vitamin B₆, 22.3 µg.

*SID = standardized ileal digestible.

A&M University (College Station, TX). While the calculated composition of major dietary nutrients is listed in Table 1, the analyzed composition of dietary nutrients including AA is reported in Table 2.

Growth Performance Measurement and Blood Sample Collection

Pigs were allowed ad libitum access to fresh water and the experimental diets throughout the trial, which lasted for a total of 8 weeks. All the pigs, feeders, and waterers were inspected two to three times daily (0600 to 1900 h). Pigs' BW and feed intake were measured at the beginning and the end of the 8 week period for calculation of average daily gain (ADG), average daily feed intake (ADFI) and gain to feed (G:F) ratio. All the experimental protocols including caring, handling, and treatment of pigs were approved by the Mississippi State University Institutional Animal Care and Use Committee.

At the end of the 8-week feeding trial, blood samples (approximately 10 mL/pig) were collected by jugular

venipuncture of individual pigs in the early morning (between 0600 and 0800 h). Immediately before blood collection, the remaining feed in all the feeders were removed to avoid any re-pushed concentrations of nutrients or metabolites in the blood due to any immediate morning meal. Blood samples, collected in a non-fasting state resembling a typical swine industry setting, were kept on ice immediately after collection until plasma was separated within 60 min by centrifugation at $800 \times g$ and 4°C for 16 min. Plasma sample aliquots were then stored at -80°C until the analyses of AA, nutrient metabolites, and growth-related hormones.

Laboratory Analyses

The concentrations of plasma free AA (except for proline and cysteine) were determined using a high-performance liquid chromatography (HPLC) method (Wu, 1994; Dai et al. 2014). Briefly, after a pre-column derivatization of plasma AA with o-phthalaldehyde, the samples were separated through a Supelco 3- μm reversed-phase C18 column (46 mm $\times$ 150 mm, i.d.) guarded by a Supelco 40- μm reversed phase C18 column (46 mm $\times$ 50 mm, i.d.). The HPLC mobile phase consisted of solvent

A (0.1 M sodium acetate/0.5% tetrahydrofuran/9% methanol; pH 7.2) and solvent B (methanol), with a combined total flow rate of 1.1 mL/min. A gradient program with a total running time of 49 min was developed for a satisfactory AA separation.

Batch analysis using the automated ACE Alera Clinical Chemistry System (Alfa Wassermann, West Caldwell, NJ) was performed for determination of six plasma metabolites with six respective ACE reagents (Alfa Wassermann) at the College of Veterinary Medicine Diagnostic Laboratory of Mississippi State University. The batch analyses involved respective enzymatic reactions, followed by bichromatic measurements of respective reaction products at different wavelengths. The six metabolites analyzed included total protein, albumin, plasma urea nitrogen, glucose, triglycerides, and total cholesterol. Detailed assay procedures can be found in Regmi et al. (2018).

For the plasma concentrations of the selected hormones, three commercial ELISA kits were employed for the measurements, as previously reported by Wang et al. (2017). The porcine growth hormone (GH, a.k.a. somatotropin) ELISA kit (Catalog number SEA044P0) was purchased from the Cloud-Clone Corp. (Wuhan, China), The porcine insulin ELISA kit (Catalog number ENZ-KIT 141-0001) and the human IGF-1 ELISA kit (Catalog number ADI-900-150) were purchased from the Enzo Life Sciences (Farmingdale, NY). The manufacturers' instructions for using these ELISA kits were followed in our laboratory for the analyses.

Statistical Analysis

Data were analyzed by Student's *t*-test using the TTEST procedure of SAS program (Version 9.3; SAS Institute, Inc., Cary, NC). The dietary lysine level was the main effect and pigs were experiment units. Parameter values were presented as means $\pm$ standard deviation. When $P \leq 0.05$, the difference was considered to be significant, and when $0.05 < P \leq 0.10$, the difference was considered to have a tendency to be different.

Results and Discussion

Growth Performance

The results of the growth performance of the growing pigs in response to dietary lysine restriction are given in Table 3. There was no difference ($P = 0.90$) in pig's initial BW between the LDD and LAD groups. At the end of the trial, the final BW, ADG, and G:F ratio were significantly decreased ($P < 0.05$) in the pigs fed

Table 2. Analyzed nutrient composition (% or as indicated) of the experimental diets

Item	Diet ¹	
	LDD	LAD
<i>Proximate analysis²</i>		
Dry matter, %	87.8	87.9
Gross energy, kcal/kg	3946	3925
Crude protein, %	15.2	15.2
Crude fat, %	3.26	3.25
Crude fiber, %	1.82	1.78
Neutral detergent fiber, %	13.5	11.3
Acid detergent fiber, %	2.58	2.28
Ash, %	4.22	3.97
<i>Amino acid (nmol/mL)</i>		
Alanine	0.836	0.815
Arginine	1.015	0.987
Aspartic acid	1.579	1.524
Cysteine	0.268	0.268
Glutamic acid	2.789	2.695
Glycine	0.667	0.642
Histidine	0.396	0.383
Isoleucine	0.672	0.643
Leucine	1.369	1.329
Lysine	0.818	1.048
Free lysine	0.158	0.151
Methionine	0.337	0.323
Met + Cys	0.605	0.591
Phenylalanine	0.788	0.766
Proline	0.937	0.930
Serine	0.781	0.761
Threonine	0.680	0.659
Tyrosine	0.392	0.377
Valine	0.745	0.714
Tryptophan	0.195	0.193

¹LDD, the lysine-deficient diet; LAD, the lysine-adequate diet. The calculated total lysine contents (as-fed basis) in LDD and LAD were 0.81% and 1.12%, respectively.

²Proximate and energy analyses were conducted at the Essig Animal Nutrition Laboratory, Mississippi State University (Starkville, MS). Amino acid composition was analyzed at Ajinomoto Heartland, Inc. (Chicago, IL).

Table 3. The growth performance of the growing pigs fed a lysine-deficient vs. a lysine-adequate diet

Item	Diet [*]		P-value [†]
	LDD	LAD	
Initial BW, kg	22.7 $\pm$ 2.37	22.5 $\pm$ 1.88	0.902
Final BW, kg	76.0 ^a $\pm$ 2.00	80.3 ^b $\pm$ 2.56	0.009
ADG, kg/day	0.95 ^a $\pm$ 0.04	1.03 ^b $\pm$ 0.04	0.004
ADFI, kg/day	2.26 $\pm$ 0.14	2.31 $\pm$ 0.12	0.524
G:F ratio	0.42 ^a $\pm$ 0.02	0.45 ^b $\pm$ 0.02	0.038

^{*}LDD, the lysine-deficient diet; LAD, the lysine-adequate diet. Each value is a Mean $\pm$ Standard deviation ($n = 6$); ADG, average daily gain; ADFI, average daily feed intake; G:F ratio, gain to feed ratio.

[†]P-value was obtained from Student's *t*-test.

^{a,b}Means within a row that have different superscripts differ ($P < 0.05$).

LDD than fed LAD, and there was no difference ($P = 0.52$) in the ADFI between the two groups of pigs.

As expected, the dietary lysine restriction did not affect the ADFI, but the G:F ratio and, therefore, the ADG and final BW of the pigs. This result is consistent with numerous previous reports (O'Connell et al., 2006; Yang et al., 2008; Jin et al., 2010; Kim et al., 2011; Taylor et al., 2012, 2015), with all concluded that the pigs grew slowly and feed utilization was less efficient when fed reduced levels of dietary lysine. The reduced growth performance observed in this study must be the consequence of pigs' poor utilization of dietary nutrients and, especially the proteinogenic AA, for body protein biosynthesis (Wu et al., 2014; Liao et al., 2015; Wang et al., 2015) since the skeletal muscle is the largest organ, as well as the largest protein pool, in animal body (te Pas et al., 2004; Li et al., 2016).

Plasma AA profile

The effect of dietary lysine restriction on the concentrations of plasma-free AA of the pigs is given in Table 4. As expected, the plasma lysine concentration was decreased ($P < 0.01$) in pigs fed LDD vs. LAD. Besides lysine, the plasma concentrations of methionine, leucine (two essential AA), and tyrosine (a nonessential AA) were also decreased ($P < 0.05$) in pigs fed LDD. The plasma concentration of arginine (an essential AA) tended to be decreased ($P < 0.10$) by LDD. Meanwhile, the concentration of β -alanine (a non-dietary, non-proteinogenic AA) was increased ($P < 0.01$) in pigs fed LDD. There were no differences ($P > 0.15$) in the concentrations of all other AA between the two groups of pigs.

Table 4. The plasma concentrations of free amino acids of the pigs fed a lysine-deficient vs. a lysine-adequate diet

	Diet†		
Amino acid, nmol/mL*	LDD	LAD	P-value‡
Essential amino acids			
Lysine	70.8 ^a ± 18.1	126.3 ^b ± 16.6	0.002
Arginine	96.5 ± 11.5	116.0 ± 18.5	0.052
Leucine	137.1 ^a ± 4.9	144.2 ^b ± 5.5	0.035
Methionine	29.5 ^a ± 2.3	33.3 ^b ± 0.5	0.003
Histidine	70.9 ± 2.8	70.0 ± 8.9	0.779
Threonine	147.3 ± 28.9	142.6 ± 14.8	0.730
Valine	188.3 ± 12.0	189.5 ± 20.7	0.900
Isoleucine	91 ± 5.3	92 ± 8.3	0.833
Phenylalanine	67.11 ± 3.4	59.7 ± 10	0.119
Tryptophan	68.5 ± 3.6	69.6 ± 3.6	0.622
Nonessential amino acids			
Tyrosine	70.4 ^a ± 6.8	82.85 ^b ± 7.8	0.014
β-alanine	47.30 ^a ± 9.7	30.1 ^b ±7.4	0.005
Serine	79.6 ± 5.5	76.8 ± 8.1	0.497
Taurine	64.37 ± 12.0	58.66 ± 14.5	0.475
Ornithine	85.5 ± 10.9	90.8 ± 14.5	0.491
Asparagine	32.2 ± 12.3	36 ± 3.0	0.482
Glutamine	408.8 ± 68.7	432.4 ± 59.0	0.538
Glycine	813.8 ± 130.0	742.2 ± 90.74	0.294
Alanine	197.7 ± 29.3	193.5 ± 33.2	0.821
Aspartic acid	5.4 ± 1.7	4.4 ± 1.0	0.240
Glutamic acid	72.9 ± 19.0	57.8 ± 14.5	0.151
Citrulline	97.5 ± 62.2	64.9 ± 10.0	0.233

*Plasma amino acid concentration. Each value is a mean ± standard deviation ($n = 6$).

†LDD, the lysine-deficient diet; LAD, the lysine-adequate diet.

‡P-value obtained from Student's t-test.

The decreased plasma concentration of lysine must be due to the restricted dietary lysine supply, which is consistent with the result of Regmi et al. (2016), who investigated the effects of three levels (deficient, adequate, and excess) of dietary lysine on plasma AA profile in finishing pigs and found a significant decrease in the plasma lysine concentration in the pigs fed a lysine deficient diet. Similarly, Zeng et al. (2013) and Roy et al. (2000) also reported that the plasma lysine concentration was linearly decreased with the decreased dietary lysine levels. As is known, dietary proteins or free AA are the primary source of free AA circulated with the blood stream to other parts of the body for metabolic utilization, especially for muscle protein synthesis (Fabian et al., 2002; Liao et al., 2015, 2018; Morales et al., 2015), and lysine serves as an important building block for protein synthesis.

The result of the reduced plasma concentration of methionine in the LDD pigs of this study partially agrees with Morales et al. (2015), who reported that lysine deficiency numerically decreased plasma concentration of methionine in growing pigs. Methionine is the second or third limiting AA in typical swine diets and one functional AA that plays important roles in nutrient metabolism, protein turnover, and immune response (Wu, 2010, 2013; Yang et al., 2019). For example, protein synthesis is universally initiated with methionine, which is encoded by a universal initiation codon AUG. Thus, the reduced plasma methionine level may suppress protein synthesis via halting protein synthesis initiation (Kozak, 1992; Drabkin and RajBhandary, 1998), which might be one of the molecular bases for the poor ADG in growing-finishing pigs (Chung and Baker, 1992; Wu, 2010; Yang et al., 2019). Hiramoto et al. (1990) reported that dietary deficiency of both methionine and lysine can reduce the protein synthesis rate by 36% in laying hens. Thus, a low plasma concentration of methionine in addition to lysine can further worsen pigs' growth performance.

Plasma concentration of another essential AA leucine was decreased in pigs fed the LDD. Similar trend of decrease in plasma concentration of leucine was reported by Morales et al. (2015), who found that the plasma leucine concentration was decreased (numerically) in growing pigs fed a lysine deficient diet relative to a lysine adequate diet. Leucine not only serves as a building block for protein synthesis, but also serves as an important cell-signaling molecule for the mammalian target of rapamycin (mTOR) pathway related to the protein synthesis in various tissues including skeletal muscle (Drummond and Rasmussen, 2008), adipose (Lynch et al., 2002), placental, and mammary gland (Rezaei et al., 2016). In addition, leucine can also enhance energy homeostasis through augmenting mitochondrial biogenesis and fatty acid oxidation to support protein synthesis (Duan et al., 2016). Thus, the decreased plasma leucine concentration in the LDD pigs of this study might participate to the poor growth performance of the pigs.

The plasma concentration of β -alanine (a non-proteinogenic AA) was increased in the LDD pigs. To date, nothing is known about the role of lysine on the endogenous biosynthesis of β -alanine in pigs. However, it is well known that β -alanine has many important biochemical functions, such as the synthesis of carnosine. Carnosine is a buffer molecule to neutralize lactic acid from heavy muscle activity and maintain the intracellular pH in skeletal muscle (Culbertson et al., 2010). Therefore, it would be interesting to conduct further research based on the concept that high plasma concentration of β -alanine in response to dietary lysine restriction may promote the synthesis of carnosine in skeletal muscle, which would further help to maintain muscle pH and minimize the yield of PSE (pale, soft,

and exudative) pork in the industry (Bowker et al., 2000; Kim et al., 2016).

In short, although the metabolic reason why the plasma concentrations of methionine, leucine, and tyrosine were reduced and why the plasma concentration of β -alanine was increased are not clear at this moment, we at least know that like lysine, these AA (including arginine, but not β -alanine) are not only the building blocks for, but also the important variegate modulators of body protein synthesis (Blachier et al., 2013).

Plasma Concentrations of Nutrient Metabolites

As given in Table 5, there was a significant decrease ($P < 0.02$) in the plasma total protein concentration in pigs fed the LDD vs. the LAD, whereas no differences ($P > 0.37$) were observed in the plasma concentrations of other metabolites between the two groups of pigs. In agreement with this result, some previous research also reported that the plasma total protein concentrations were decreased in pigs fed lysine deficient diets (Kamalakar et al., 2009; Yang et al., 2009; Zeng et al., 2013). As is known, plasma total protein content is a good indicator of protein metabolism in pigs, and the reduction of total protein concentration in this study suggests that the capacity of hepatic or whole-body protein synthesis was negatively affected by the dietary lysine restriction. In terms of plasma albumin, a disagreement result was observed by Kamalakar et al. (2009), who reported that the plasma albumin concentration was decreased in growing pigs fed lysine deficient diets (60% and 80% of NRC recommendation). This discrepancy might be due to the different magnitudes of deficiency and length of feeding period between the two studies.

In theory, dietary lysine deficiency would increase the plasma concentration of urea nitrogen because a rapid depletion of lysine can halt body protein synthesis and the other AA would be catabolized to produce ammonia and urea, and so on and, thus, plasma urea nitrogen is usually used as an indicator to assess the efficiency of body protein synthesis (Zeng et al., 2013; Regmi et al., 2018). In this study, although not statistically significant ($P > 0.05$), the plasma urea nitrogen concentration was numerically higher in pigs fed the LDD than LAD (Table 5). In the literature, similar results were reported by Kamalakar et al. (2009) and Jin et al. (2010) when pigs were fed different levels of dietary lysine.

There were no differences ($P = 0.92$) in the plasma glucose concentration between the two dietary treatments (Table 5), which suggests that the glucose or energy metabolism was not affected by dietary lysine restriction. This result is consistent with previous reports stated that different levels of dietary

lysine did not affect the plasma concentration of glucose (Mule et al., 2006; Yang et al., 2008; Kamalakar et al., 2009; Regmi et al., 2018). Although the lysine content of LDD was lower than that of LAD, the calculated net energy content of LDD was the same as that of LAD (Table 1), and this might explain why the plasma glucose concentration was not changed. Gannon and Nuttall (2010) also suggested that although most AA may modestly change the insulin and glucagon areas of response, these AA do not significantly change the glucose concentration in normal young subjects.

Theoretically, dietary lysine restriction would limit the use of other AA for protein synthesis, and these surplus AA could offer more carbon skeleton for lipid synthesis in the body. However, in this study there was no difference ($P > 0.05$) in the plasma concentrations of triglycerides and total cholesterol (Table 5), indicating that the dietary lysine restriction did not affect the plasma lipid homeostasis. In fact, this finding is consistent with some previous reports (Mule et al., 2006; Kamalakar et al., 2009), showing that the dietary lysine level did not affect the concentrations of triglycerides and cholesterol.

Plasma Concentrations of Anabolic Hormones

As given in Table 6, there were no differences ($P > 0.42$) in the plasma concentrations of GH, insulin, and IGF-1 between the two dietary treatments, indicating that the plasma levels of these anabolic hormones in growing pigs were not affected by the dietary lysine restriction. Similar result was reported by Roy et al. (2000), who reported that dietary lysine levels did not affect the plasma concentrations of GH and IGF-1 in growing pigs. Wang et al. (2017) found that there was no effect of dietary lysine levels on the plasma concentrations of GH and insulin in late-stage finishing pigs.

It has been well known that dietary nutrients exert their functions through numerous nutrient-metabolic and cell-signaling pathways (Hasan et al., 2019). Of these, the GH/IGF-1 axis is one of the primary regulatory systems for animal growth and this axis is sensitive to animal nutritional status (Brameld et al., 1998; Breier, 1999; Jiang and Ge, 2014). Katsumata et al. (2002) reported that dietary lysine deficiency reduced the plasma IGF-1 concentration by 52% in growing pigs, whereas Wang et al. (2017) reported that the plasma IGF-1 concentration was decreased when finishing pigs were fed either a lysine deficient or a lysine excess diet.

Additionally, it is also known that different AA have different potencies of insulinotropic property, and lysine or arginine appeared to be the most potent one (Floyd et al., 1966; Fajans et al., 1969; Wang et al., 2017). Ren et al. (2007) reported that the plasma insulin concentration was increased when growing

Table 5. The plasma concentrations of nutrient metabolites in growing pigs fed a lysine-deficient vs. a lysine-adequate diet*

Parameter (mg/dL)	Diet*		P-value†
	LDD	LAD	
Total protein	5.9 ^a ± 0.25	6.2 ^b ± 0.15	0.019
Albumin	3.9 ± 0.25	3.9 ± 0.25	0.909
Urea nitrogen	9.5 ± 2.9	8.2 ± 2.04	0.377
Glucose	93.9 ± 4.8	94.2 ± 6.7	0.922
Triglycerides	35.9 ± 5.15	38.9 ± 7.0	0.416
Total cholesterol	86.5 ± 12.7	88.0 ± 5.3	0.794

*LDD, the lysine-deficient diet; LAD, the lysine-adequate diet. Each value is a mean ± standard deviation (n = 6).

†P-value obtained from Student's t-test.

^{a,b}Means within a row that have different superscripts differ ($P < 0.05$).

Table 6. The effects of lysine restriction on growth related hormones of growing pigs*

Item	Diet*		P-value†
	LDD	LAD	
GH, ng/mL	1.07 ± 0.24	1.12 ± 0.30	0.648
Insulin, μ U/mL	12.02 ± 3.01	10.05 ± 4.42	0.421
IGF-1 [‡] , ng/mL	286.9 ± 79.53	289.7 ± 65.20	0.673

*LDD, the lysine-deficient diet; LAD, the lysine-adequate diet. Each value is a mean ± standard deviation (n = 6).

†P-value obtained from Student's t-test.

‡IGF, insulin like growth factor.

^{a,b}Means within a row that have different superscripts differ ($P < 0.05$).

pigs were fed a lysine excess diet. Roy et al. (2000) reported that although the growing barrows fed diets containing 0.45% and 0.75% lysine showed no difference in plasma insulin concentration, the plasma insulin concentration tended to increase when pigs were fed 0.98% lysine.

The unchanged plasma concentrations of GH, IGF-1, and insulin observed in this study suggest that the negative effect of dietary lysine restriction on animal growth (Table 3) may result from 1) the lack of lysine as a building block for protein synthesis, and 2) some shifts in AA metabolism that led to a reduction in the plasma supply of methionine, leucine, and tyrosine (Liao et al., 2018). Nevertheless, the reduced growth performance or reduced rate of body protein synthesis (as indicated in Tables 3 and 5) may not be mediated by the GH/IGF-1 axis.

Conclusions

Dietary lysine restriction reduced the plasma concentrations of three essential AA, lysine, methionine, and leucine, as well as one nonessential AA, tyrosine, but increased that of β -alanine, in the young growing pigs. The lysine restriction reduced the plasma concentration of total protein, but not the plasma concentrations of three anabolic hormones, GH, insulin, and IGF-1. These results confirmed that the lack of lysine as a protein building block must be the primary reason for the compromised G:F ratio and ADG of pigs resulted from dietary lysine restriction. These results, especially that of total plasma protein, also suggest that the metabolic reduction in the concentrations of methionine, leucine, and tyrosine might further contribute to the compromised growth performance through some cell signaling and metabolic pathways that regulate AA metabolism and protein synthesis. However, the compromised growth performance may not involve the GH/IGF-1 axis pathway. Furthermore, whether or not the increased plasma concentration of β -alanine in lysine-deficient pigs can reduce the occurrence of PSE pork warrants further investigation.

Conflict of interest statement

Authors declare that they have no conflicts of interest.

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