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Complete List of Authors:	Wang, Taiji; Mississippi State University, Department of Animal and Dairy Sciences Crenshaw, Mark; Mississippi State University, Department of Animal and Dairy Sciences Regmi, Naresh; Mississippi State University, Department of Animal and Dairy Sciences Rude, Brian ; Mississippi State University, Department of Animal and Dairy Sciences Hasan, M. Shamimul; Mississippi State University, Department of Animal and Dairy Sciences Sukumaran, Anuraj ; Mississippi State University, Department of Animal and Dairy Sciences Dinh, Thu; Mississippi State University, Department of Animal and Dairy Sciences Liao, Shengfa; Mississippi State University, Department of Animal and Dairy Sciences
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Effects of dietary lysine level on the content and fatty acid composition of intramuscular fat in late-stage finishing pigs

Taiji Wang, Mark A. Crenshaw, Naresh Regmi, Brian J. Rude, M. Shamimul Hasan, Anuraj T. Sukumaran, Thu Dinh, and Shengfa F. Liao*

Department of Animal and Dairy Sciences, Mississippi State University, Mississippi, USA

*Correspondence:

Shengfa F. Liao, PO Box 9815, Mississippi State University, Mississippi State, MS 39762, USA.

Tel: +1 662 3257318; Fax: +1-662-3258873; E-mail: s.liao@msstate.edu

Abstract

This study was conducted to investigate how dietary lysine level affects the intramuscular fat (IMF) content and fatty acid (FA) composition in late-stage finishing pigs. Nine crossbred barrows (94.4 ± 6.7 kg BW) were randomly allotted to three treatment groups ($n = 3$). Three corn and soybean-meal based diets were formulated to meet the NRC (2012) requirements for various nutrients except for lysine, whose concentrations were 0.43%, 0.71%, and 0.98% (as-fed basis) for Diets 1 (lysine-deficient), 2 (lysine-adequate), and 3 (lysine-excess), respectively. After 5 weeks of ad libitum access to diets, pigs were harvested and *longissimus dorsi* samples were collected. The IMF content and FA composition of the samples were analyzed by gas chromatography. Results showed that the IMF content of the muscle was increased linearly ($P < 0.05$) with decreasing dietary lysine level from 0.98% to 0.43%. Dietary lysine level altered the composition of FA, especially the unsaturated FA, in the muscle. Particularly, the percentages of C18:1 n-9 and total mono-unsaturated FA were higher, while the percentages of C18:2 n-6 and total poly-unsaturated FA were lower, in the muscle of the pigs fed Diet 1. Collectively, dietary lysine deficiency increased the proportion of mono-unsaturated FA and decreased the proportion of poly-unsaturated FA, which may benefit pork palatability.

Key words: lysine, fatty acid, intramuscular fat, skeletal muscle, pig (finishing)

Abbreviations: ANOVA, analysis of variance; BW, body weight; FA, fatty acid or fatty acids; FAME, fatty acid methyl esters; GC, gas chromatography; GLM, general linear model; IMF, intramuscular fat; MUFA, mono-unsaturated fatty acid; PUFA, poly-unsaturated fatty acid; SCD, stearoyl-CoA desaturase; SFA, saturated fatty acid; SI, saturation index; UFA, unsaturated fatty acid

Introduction

Human consumption of animal meat and meat products continues to grow globally (Wu et al. 2014), and along with this trend is the increased concerns about meat quality, especially the content and fatty acid (FA) composition of the intramuscular fat (IMF; a.k.a. marbling) in the skeletal muscle (Bessa et al. 2013). While pork quality is closely related to the IMF content (Fernandez et al. 1999; Fortin et al. 2005), the FA composition of IMF plays very important roles not only in pork processing quality but also in pork nutritional value for consumers. Excessive consumption of animal fat, especially, the saturated FA (SFA), increases the risks of obesity and metabolic disorders in humans (Aranceta and Perez-Rodrigo, 2012; Kratz et al. 2013), whereas some unsaturated FA (UFA) including mono-unsaturated FA (MUFA) and poly-unsaturated FA (PUFA) are considered beneficial for human health (FAO, 2010). Therefore, a challenge in the pork industry is to produce pork with an optimal IMF content and appropriate FA composition to ensure a superior pork palatability and processing capability (bacon slicing, shelf life, etc.) while minimizing any health concerns of the consumers (Fortin et al. 2005).

Animal nutritional strategies can be applied to swine husbandry to enhance pork quality by manipulating the IMF content and its FA composition in the skeletal muscle (Katsumata, 2011). It has been reported that the reduction of dietary protein level increased the IMF content in pigs (Castell et al. 1994; da Costa et al. 2004; Doran et al. 2006) and pigs fed high leucine diets deposited more IMF in the *longissimus dorsi* muscle (Hyun et al. 2003). Furthermore, different dietary oil sources containing different FA's could also influence the FA composition of pork (Wood et al. 2004a, 2008).

In our previous study, dietary lysine affected the expression of several key genes, such as the gene encoding stearoyl-CoA desaturase (SCD; EC 1.14.19.1) that is related to FA synthesis in skeletal muscle (Wang et al. 2017). Catalyzing a rate-limiting step in the conversion of SFA to the corresponding MUFA, SCD plays a key role in altering IMF composition in pigs (da Costa et al. 2004; Doran et al. 2006; Bessa et al. 2013). However, whether or not the observed change in SCD gene expression caused by the dietary lysine level will lead to a change in IMF composition is unknown. Thus, this study was conducted to evaluate the effects of dietary lysine level on the IMF content and its FA composition in *longissimus* muscle of late-stage finishing pigs.

Materials and Methods

Animal Trial and Sample Collection

All the experimental protocols involving care, handling, and treatment of pigs were approved by Mississippi State University Institutional Animal Care and Use Committee. A total of 9 crossbred (Large White $\times$ Landrace) barrows with an average initial body weight (BW) of 94.4 ± 6.7 kg (mean $\pm$ SD) were purchased from a local commercial swine farm and then housed in an environmentally controlled swine barn at the Leveck Animal Research Center of Mississippi State University. All barrows from the same commercial dam and sire lines were randomly selected from a pool of mixed litters. In the swine barn, these barrows were randomly assigned to nine individual pens, and then assigned to three dietary treatment groups according to a completely randomized experimental design. Each treatment group consisted of three pen replicates ($n = 3$) with one pig per pen.

A corn and soybean meal based diet (Diet 1; a lysine-deficient diet) was formulated to meet or exceed the NRC (2012) recommended requirements for various nutrients including crude protein and essential amino acids but not for lysine. Diet 2 (a lysine-adequate diet) and Diet 3 (a

lysine-excess diet) were formulated by adding L-lysine monohydrochloride (Archer Daniels Midland Co., Quincy, IL, USA) to Diet 1 at the expense of corn at rates of 0.35% and 0.70%, respectively (Table 1). The total lysine content (calculated, as-fed basis) of Diets 1, 2, and 3 were 0.43%, 0.71%, and 0.98% , respectively. To confirm the contents of major nutrients, duplicate samples of the three diets were submitted to the Essig Animal Nutrition Laboratory at Mississippi State University for proximate analysis, and to Dr. Guoyao Wu’s laboratory at Texas A&M University (College Station, TX, USA) for amino acid analysis, and the results are shown in Table 2.

The feeding trial lasted five weeks, during which the pigs were allowed ad libitum access to the experimental diets (Diet 1, 2 or 3) and fresh water. All the pigs, feeders, waterers, and room temperature were checked 2 to 3 times daily (between 06:00 to 17:00 o'clock). At the end of the trial, pigs were harvested in the Meat Science and Muscle Biology Laboratory of Mississippi State University, and *longissimus dorsi* muscle samples were collected between the tenth and twelfth ribs, immediately snap-frozen in liquid nitrogen, and then stored at -80°C until analysis of FA.

Fatty Acid Analysis

The IMF of *longissimus dorsi* muscle was extracted and derivatized using a gas chromatography (GC) method described by O'Fallon et al. (2007). Briefly, muscle samples were trimmed of all external fat and connective tissues. Approximately 1 g of each trimmed sample was weighed into a 20-mL borosilicate vial with Teflon®-lined screw-cap, to which 1.0 mL of 1.5-mg mL⁻¹ tridecanoate methyl ester (13:0 in methanol) internal standard, 0.7 mL of 10-N KOH, and 5.3 mL of methanol were added. The tube was capped tightly and incubated in a 55°C water bath for 1.5 h with vigorous hand-shaking every 20 min to liberate FA. After cooling the

tube in cold water, 0.58 mL of 24-N H_2SO_4 was added. The solution was mixed by inversion of the tube. The tube was then incubated again in a 55°C water bath for another 1.5 h to synthesize FA methyl esters (FAME). After that the tube was cooled in cold water, and the FAME were extracted in 3 mL of hexane for 5 min on a multi-tube vortex. The tube was centrifuged for 5 min, and the hexane layer containing the FAME mixture was transferred into an amber 2-mL GC vial with a Teflon[®]-lined screw-cap. Vials were then capped tightly and stored at -20°C until the GC determination of FA composition.

The FA composition was determined by an Agilent 7890A GC system equipped with a HP-88 capillary column (30 m × 0.25 mm × 0.20 µm), a flame ionization detector, an autosampler, and a split/splitless injector (Agilent Technologies Inc., Santa Clara, CA, USA). The FAME were separated in a 20-min temperature-gradient program with hydrogen as the carrier gas flowing at a constant rate of 1.5 mL min⁻¹. The injector was set at a constant 250°C with 100 : 1 split ratio. The flame ionization detector was set at 300°C, 30 mL min⁻¹ of hydrogen flow, and 400 mL min⁻¹ of air flow. Data was collected at 100 Hz. Fatty acid methyl esters were identified by comparing their retention times with authentic FAME standards and calculated by an internal standard calibration method.

The gravimetric concentration of each FA (mg g⁻¹ of muscle) was calculated according to Dinh et al. (2010) with correction by the molecular weight difference between FAME and their corresponding FA. The total FA concentration (mg g⁻¹ of muscle) was used as an estimate of the IMF content (Wood et al. 2013). The normalized percentage of each individual FA (% of total FA) was used to represent the proportion of a given FA content relative to the total FA content. The FA saturation index (SI) was calculated as a ratio of total SFA to total UFA (including MUFA and PUFA) concentration (Dinh et al. 2010). The ratio of the C16:1 n-7 and C18:1 n-9

concentration to the total concentration of C16:0, C16:1 n-7, C18:0, and C18:1 n-9 was calculated as a SCD activity index (Yang et al. 1999; Miyazaki et al. 2003; Doran et al. 2006; Dinh et al. 2010).

Statistical Analysis

Dietary treatment effects on the IMF content, the FA concentration, and the FA percentages, as well as the SI and SCD activity index of the skeletal muscle were all subjected to analysis of variance (ANOVA) for a completely randomized experimental design. The ANOVA analyses were performed using the general linear model (GLM) procedure of SAS (SAS 9.4, SAS Inst. Inc., Cary, NC, USA). Relationships between dietary lysine level and the IMF concentration, as well as between dietary lysine level and FA concentrations, were determined using orthogonal polynomial contrasts for linear and quadratic effects. For the SI and SCD activity index, means were separated by protected t-test using the LSMEANS/PDIFF option in the GLM procedure. Probability (P) value less than or equal to 0.05 was considered to be significant and P value between 0.05 and 0.10 was considered to have a tendency to be significant.

Results and Discussion

Lysine Effect on Fatty Acid Concentration

The gravimetric concentrations (mg g^{-1}) of the individual and total FA in the *longissimus dorsi* muscle of the finishing pigs are presented in Table 3. First of all, the concentrations of IMF, total SFA, and total MUFA increased linearly ($P < 0.05$) with decreasing dietary lysine level from Diet 3 to Diet 1. In terms of individual SFA, the concentrations of C14:0 and C16:0 increased linearly ($P < 0.05$), whereas the concentration of C15:0 tended to decrease linearly (P

= 0.08) with decreasing dietary lysine level. The concentrations of all other individual SFA were not affected ($P > 0.11$) by the dietary lysine level. In terms of individual MUFA, the concentrations of C16:1 n-7, C18:1 n-9, and C20:1 n-9 increased linearly ($P < 0.04$) with decreasing dietary lysine level, whereas the concentrations of all other individual MUFA were not affected ($P > 0.44$) by the dietary lysine level. In terms of individual PUFA, only the concentration of C18:3 n-3 was increased quadratically ($P = 0.027$) with increasing dietary lysine level.

The increased total FA concentration along with the reduced dietary lysine level from excess to deficiency (Table 3) suggests that the IMF content of skeletal muscle can be changed by dietary lysine. Similar results were found by Katsumata et al. (2005) previously, who reported that a reduced dietary lysine level promoted an accumulation of IMF in the *longissimus dorsi* of finishing gilts. Several other studies have also shown that there were increases in the IMF content of the *longissimus dorsi* in pigs fed reduced protein (combination of lysine and other amino acids) diets (Wood et al. 2004b; Doran et al. 2006). It was reported that dietary lysine deficiency associated with reduced dietary protein content is needed to increase the IMF content of skeletal muscle of the lean (rather than fatty) genotype pigs (Madeira et al. 2013a, 2013b).

All these previous studies together with this study suggest that dietary lysine and/or protein level plays important roles in regulating the IMF content in porcine skeletal muscle. Nonetheless, although the IMF content was altered by the lysine level in the present study, the loin marbling scores did not change among the three groups of pigs fed diets containing three different levels of lysine (Wang et al. 2015). This apparent discrepancy suggests that the change in IMF content might not be dramatic enough to lead to a change in marbling appearance. Additionally, the lipids in myocytes might have been included in the analysis of IMF content

(Katsumata et al. 2005), but these lipids do not affect the muscle marbling appearance. Furthermore, previous research reported when comparing to the marbling appearance in bovine meat, pork marbling is not as visible just because of the background color of lean and softer fat in pork (Mourot and Hermier, 2001; Madeira et al. 2013b).

The present study revealed a trend that is increasing dietary lysine level reduces the IMF content in the *longissimus dorsi* muscle of finishing pigs. Contrary to this finding, feeding a normal diet supplemented with 2% leucine (Hyun et al. 2003) or 1% arginine (Ma et al. 2015) increased the IMF content of the *longissimus dorsi* in finishing pigs, although the effect of dietary threonine level on the IMF accumulation was minimal in finishing pigs (Kobayashi et al. 2012). Therefore, the underlying metabolic mechanisms through which different dietary amino acids influence IMF accumulation differently need to be further elucidated.

Lysine Effect on Fatty Acid Proportions

As percentages over the total FA content, the proportions of individual FA in the IMF of *longissimus dorsi* of the finishing pigs are presented in Table 4. Firstly, from Table 4 it can be seen that the predominant FA in the *longissimus dorsi* muscle were C18:1 n-9 (40-45%), C16:0 (25-27%), C18:0 (12-14%), and C18:2 n-6 (6-11%), irrespective of dietary treatments, and this is in agreement with the data widely reported in the literature (Mourot and Hermier, 2001). In terms of the dietary lysine effects on the proportions of FA, the percentages of C14:0 and C16:0 tended to increase linearly ($P < 0.10$), whereas the percentages of C15:0 and C17:0 tended to decrease linearly ($P \leq 0.10$) with decreasing dietary lysine level. The percentages of C18:0 and total SFA tended to increase quadratically ($P < 0.07$) with decreasing dietary lysine level.

For MUFA, while the percentages of C18:1 trans and C24:1 n-9 (nervonic acid) decrease linearly ($P < 0.04$) with decreasing dietary lysine level from Diet 3 to Diet 1, the percentages of

C18:1 n-9, C20:1 n-9, as well as total MUFA, increased linearly ($P < 0.03$) with decreasing dietary lysine level. As is known, C18:1 n-9 (oleic acid) is a predominant MUFA, which may explain why the percentage of total MUFA followed the same pattern as that of oleic acid. Similarly, Doran et al. (2006) reported that the level of total MUFA in *longissimus thoracis* of growing pigs was elevated when a diet with a reduced level of protein was fed.

For PUFA, while the percentages of C20:2, C20:3 n-6, and C20:4 n-6 tended to decrease linearly ($0.05 < P < 0.10$) with decreasing dietary lysine level, the percentages of C18:2 n-6, C18:3 n-3, as well as the total PUFA, significantly decreased linearly ($P \leq 0.014$) with decreasing dietary lysine level. As is known, both C18:2 n-6 (linoleic acid) and C18:3 n-3 (linolenic acid) are essential FA in the muscle and linoleic acid is a predominant PUFA. The result of decreasing in the proportions of linoleic and linolenic acids, as well as total PUFA, with decreasing dietary lysine level is in agreement with a previous study (Katsumata et al. 2005) that reported a reduction in PUFA, including linoleic acid, in the *longissimus dorsi* of finishing gilts fed a diet containing a low level of dietary lysine.

The data reported above indicates that dietary lysine not only influenced IMF deposition and FA concentrations, but also the *de novo* synthesis of FA (Dinh et al. 2010), as well as the FA desaturation, as evidenced by the alteration in FA proportions. It has been reported that the proportions of MUFA and PUFA are associated with the eating quality of pork products. In general, the eating quality improves as the MUFA proportion increases (Cameron and Enser, 1991) because oleic acid (C18:1 n-9) is positively associated with meat flavor. A greater concentration of oleic acid in muscle lipids has been reported to parallel with the increase in taste panel scores for tenderness and juiciness (Rhee et al. 1990). On the other hand, the eating quality improves as the proportion of PUFA decreases (Cameron and Enser, 1991), which is due to the

greater susceptibility of PUFA to produce undesired volatile compounds during the cooking process (Larick et al. 1992; Legako et al. 2015). The increased PUFA (including C18:2 n-6) content has often been reported to result in off-flavors (Dinh et al., 2010). Thus, the increased proportion of MUFA and decreased proportion of PUFA associated with a lysine-deficient diet, as reported in this study, may potentially benefit the eating quality of pork.

A lysine-deficient diet, however, was shown to have an impairing effect on animal growth performance, especially a decrease of average daily gain in pigs (Wang et al. 2015), which may make it economically unfeasible for pork producers to use this nutritional strategy (i.e., reducing dietary lysine level) to regulate IMF deposition, FA composition, and meat quality. Further studies, therefore, are needed to determine whether or not a transient use of a lysine-deficient diet followed by a lysine-adequate diet can improve the pork quality by increasing the IMF content and MUFA proportion, while at the same time taking advantage of compensatory gain phenomenon (Mule et al. 2006; Heyer and Lebret. 2007) in late-stage finishing pigs.

Lysine Effect on Fatty Acid Saturation Index and Stearoyl-CoA Desaturase Activity

Two ratios among FA were calculated for SI [i.e., SFA/(MUFA + PUFA)]; related to the degree of FA saturation (Dinh et al. 2010)] and SCD activity index [i.e., (C16:1 n-7 + C18:1 n-9)/(C16:0 + C16:1 n-7 + C18:0 + C18:1 n-9)]. As shown in Figure 1A, there was no difference ($P > 0.14$) in the SI among the three dietary treatment groups, which suggests that the SI was not affected by the dietary lysine level. As shown in Figure 1B, the SCD activity was greater ($P < 0.05$) in the pigs fed Diet 1 than that in the pigs fed Diet 2 or 3, which indicates that a high SCD activity was associated with the low dietary lysine level. This result regarding the SCD activity is supported by da Costa et al. (2004) and Doran et al. (2006), who found that the levels of SCD

mRNA and protein expression were increased in porcine skeletal muscle when the levels of dietary crude protein and lysine were low. In our previous report concerning the muscle gene expression in these pigs (Wang et al. 2017), we also found that Diet 1 (i.e., the lysine-deficient diet) significantly increased ($P < 0.05$) the mRNA abundance of the SCD gene.

Related to lipid metabolism in skeletal muscle, SCD enzyme catalyzes the conversion of SFA (primarily C16:0 and C18:0) to the corresponding MUFA (such as C16:1 and C18:1), and has been shown to be associated with the IMF formation in pigs (da Costa et al. 2004; Doran et al. 2006; Bessa et al. 2013). As suggested by the SCD gene expression data (Wang et al. 2017), together with the IMF content and FA composition data obtained from this study, it seems that a greater activity of adipogenesis might be the reason for the increased IMF content in pigs fed the lysine-deficient diet. The SCD activity indices reported here reflected the SCD gene expression data on the same animals (Wang et al. 2017), and further indicate that SCD might be a critical enzyme for regulation of FA biosynthesis and IMF content in these pigs.

Conclusions

The results of this study showed that the IMF content of *longissimus dorsi* muscle of late-stage finishing pigs increased linearly along with the reduced dietary lysine concentration. Dietary lysine at different concentrations altered the composition of FA, especially the UFA, in the skeletal muscle of pigs. In particular, the percentage of C18:1 n-9 (oleic acid) was higher, while the percentage of C18:2 n-6 (linoleic acid) was lower, in the *longissimus* muscle of pigs fed a lysine-deficient diet. Consequently, dietary lysine deficiency can increase the proportion of MUFA and decrease the proportion of PUFA, which could potentially benefit the palatability and processing quality of pork products. Further studies, however, are needed to investigate a transient use of a lysine-deficient diet to improve pork eating quality and later on to provide the

pigs with an opportunity for compensatory gain so that their overall growth performance is not impaired.

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Tables

Table 1. Compositions of three experimental diets fed to the finishing pigs (as-fed basis).^a

	Diet 1	Diet 2	Diet 3
<i>Ingredients, %</i>			
Corn	90.844	90.494	90.144
Soybean meal	6.400	6.400	6.400
Canola oil	0.800	0.800	0.800
L-Lysine-HCl (98.5%)	0.000	0.350	0.700
DL-Methionine (99%)	0.040	0.040	0.040
L-Threonine (98.5%)	0.090	0.090	0.090
L-Tryptophan (99%)	0.035	0.035	0.035
Limestone	0.650	0.650	0.650
Dicalcium phosphate	0.900	0.900	0.900
Salt	0.200	0.200	0.200
Mineral premix ^b	0.033	0.033	0.033
Vitamin premix ^c	0.008	0.008	0.008
Total	100.000	100.000	100.000
<i>Composition^d</i>			
Metabolizable energy, kcal/kg	3,319	3,323	3,326
Crude protein, %	10.45	10.75	11.05
Lysine, total, %	0.43	0.71	0.98
Methionine, total, %	0.24	0.24	0.24
Threonine, total, %	0.50	0.50	0.50
Tryptophan, total, %	0.14	0.14	0.14
Ca, total, %	0.46	0.46	0.46
P, total, %	0.43	0.43	0.43

^aDiets 1, 2, and 3 were formulated to contain dietary lysine at 0.43%, 0.71%, and 0.98% (as-fed basis), respectively, of which Diets 2 and 3 were formulated by adding 0.35% and 0.70% L-lysine-HCl (Archer Daniels Midland Co., Quincy, IL, USA) to Diet 1 at the expense of corn.

^bThe mineral premix contained 13.2% Ca, 1.0% Cu, 8.0% Fe, 5.0% Mn, 10.0% Zn, 500 ppm I, and 300 ppm Se.

396 ^cEach kilogram of vitamin premix contained 22.05 million IU vitamin A, 3.31 million IU
397 vitamin D3, 66,138 IU vitamin E, 88 mg vitamin B12, 220 mg biotin, 8,818 mg menadione,
398 15,432 mg riboflavin, 61,728 mg d-pantothenic acid, and 88,183 mg niacin.
399 ^dCalculated major nutrients including energy.

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Table 2. Analyzed amino acid contents (as-fed basis) of three experimental diets fed to the finishing pigs.^{a,b}

Nutrient and Energy	Experimental Diet		
	Diet 1	Diet 2	Diet 3
Dry matter, %	87.10	87.10	87.10
Gross energy, kcal/kg	3,663	3,608	3,559
Crude protein, %	9.77	10.60	10.86
<i>Individual Amino Acids, %</i>			
Lysine	0.42	0.70	1.01
Aspartate	0.98	0.97	0.98
Asparagine	0.74	0.75	0.75
Glutamate	1.01	1.03	1.03
Glutamine	1.42	1.44	1.43
Serine	0.53	0.52	0.54
Histidine	0.34	0.33	0.34
Glycine	0.61	0.62	0.62
Threonine	0.50	0.51	0.50
Arginine	0.73	0.74	0.74
Alanine	0.81	0.83	0.82
Tyrosine	0.52	0.52	0.53
Tryptophan	0.14	0.13	0.14
Methionine	0.25	0.25	0.26
Valine	0.65	0.66	0.65
Phenylalanine	0.66	0.65	0.66
Isoleucine	0.52	0.52	0.53
Leucine	1.42	1.44	1.45
Cysteine	0.26	0.26	0.30
Proline	1.08	1.07	1.09

^aThe energy, crude protein, and dry matter contents were analyzed at the Essig Animal Nutrition Laboratory, Mississippi State University (Starkville, MS, USA). The amino acid contents were analyzed by using the high-performance liquid chromatography methods (Dai et al. 2014) at Dr. Guoyao Wu's laboratory, Texas A&M University (College Station, TX, USA). All values are presented on as-fed basis.

^bThe slightly different levels of crude protein among the three diets was because of the different levels of dietary lysine.

Table 3. Effect of dietary lysine at three different levels on the fatty acid (FA) concentration (mg g⁻¹) of *longissimus dorsi* in late-stage finishing pigs.^a

Item	Experimental Diet			SEM	P value ^b	
	Diet 1	Diet 2	Diet 3		Linear	Quadratic
<i>Individual FA^c</i>						
C10:0	0.05	0.05	0.05	0.004	0.535	0.298
C12:0	0.03	0.04	0.03	0.004	0.392	0.455
C14:0	0.49	0.37	0.25	0.069	0.047	0.964
C15:0	0.02	0.02	0.03	0.003	0.079	0.816
C16:0	9.46	8.55	6.33	0.797	0.032	0.526
C17:0	0.11	0.10	0.12	0.015	0.645	0.637
C18:0	4.26	4.41	3.12	0.432	0.112	0.225
C20:0	0.07	0.08	0.06	0.008	0.166	0.281
C14:1 n-5	0.01	0.01	0.01	0.004	0.701	0.824
C16:1 n-7	1.24	0.94	0.75	0.125	0.034	0.758
C17:1 n-8	0.10	0.09	0.10	0.010	0.879	0.270
C18:1 trans	0.17	0.23	0.20	0.028	0.436	0.290
C18:1 n-9	15.79	13.15	10.02	1.195	0.014	0.875
C20:1 n-9	0.40	0.33	0.24	0.038	0.024	0.866
C24:1 n-9	0.12	0.10	0.12	0.008	0.618	0.216
C18:2 n-6	2.14	2.54	2.45	0.157	0.200	0.252
C20:2	0.11	0.11	0.09	0.013	0.523	0.798
C18:3 n-3	0.11	0.17	0.15	0.011	0.049	0.027
C20:3 n-6	0.08	0.08	0.08	0.005	0.752	0.855
C20:4 n-6	0.55	0.56	0.59	0.026	0.432	0.701
<i>Partial sum of FA^d</i>						
SFA	14.50	13.62	9.98	1.278	0.046	0.412
MUFA	17.84	14.85	11.45	1.333	0.014	0.919
PUFA	2.99	3.45	3.36	0.181	0.197	0.267
Total FA (IMF)	35.33	31.92	24.80	2.68	0.032	0.591

^aValues were reported as least squares means.

^bP values were obtained from the orthogonal polynomial contrast test.

^cCommon name of FA: C10:0 (capric acid), C12:0 (lauric acid), C14:0 (myristic acid), C15:0 (pentadecanoic acid), C16:0 (palmitic acid), C17:0 (heptadecanoic acid), C18:0 (stearic acid), C20:0 (arachidic acid), C14:1 n-5 (myristoleic acid), C16:1 n-7 (palmitoleic acid), C17:1 n-8 (heptadecenoic acid), C18:1 trans (eliadic acid), C18:1 n-9 (oleic acid), C20:1 n-9 (gadoleic acid), C24:1 n-9 (nervonic acid), C18:2 n-6 (linoleic acid), C20:2 (eicosadienoic acid), C18:3 n-3 (α-linolenic acid), C20:3 n-6 (γ-linolenic acid), and C20:4 n-6 (arachidonic acid).

421 ^dSFA = C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0; MUFA =
422 C14:1 n-5 + C16:1 n-7 + C17:1 n-8 + C18:1 trans + C18:1 n-9 + C20:1 n-9 + C24:1 n-9;
423 PUFA = C18:2 n-6 + C20:2 + C18:3 n-3 + C20:3 n-6 + C20:4 n-6.
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Table 4. Effect of dietary lysine at three different levels on the fatty acid percentage (% total FA) of *longissimus dorsi* in late-stage finishing pigs.^a

Item	Experimental Diet			SEM	<i>P</i> value ^b	
	Diet 1	Diet 2	Diet 3		Linear	Quadratic
<i>Individual FA^c</i>						
C10:0	0.14	0.17	0.19	0.020	0.139	0.828
C12:0	0.10	0.12	0.12	0.014	0.305	0.656
C14:0	1.38	1.15	0.97	0.153	0.103	0.886
C15:0	0.06	0.08	0.13	0.022	0.062	0.558
C16:0	26.73	26.80	25.43	0.456	0.090	0.249
C17:0	0.30	0.31	0.49	0.069	0.099	0.373
C18:0	12.04	13.75	12.59	0.453	0.423	0.041
C20:0	0.21	0.23	0.22	0.013	0.543	0.234
C14:1 n-5	0.03	0.05	0.06	0.017	0.339	0.970
C16:1 n-7	3.47	3.00	3.03	0.282	0.317	0.489
C17:1 n-8	0.29	0.27	0.43	0.051	0.115	0.199
C18:1 trans	0.48	0.72	0.81	0.064	0.010	0.375
C18:1 n-9	44.80	41.08	40.23	0.890	0.011	0.236
C20:1 n-9	1.14	1.03	0.97	0.039	0.023	0.603
C24:1 n-9	0.35	0.32	0.48	0.034	0.038	0.083
C18:2 n-6	6.05	8.03	10.10	0.709	0.007	0.957
C20:2	0.30	0.33	0.39	0.032	0.097	0.654
C18:3 n-3	0.31	0.53	0.61	0.063	0.014	0.420
C20:3 n-6	0.24	0.26	0.34	0.032	0.082	0.523
C20:4 n-6	1.59	1.79	2.42	0.243	0.052	0.496
<i>Partial sum of FA^d</i>						
SFA	40.95	42.60	40.13	0.759	0.474	0.069
MUFA	50.56	46.47	46.01	0.711	0.003	0.079
PUFA	8.49	10.93	13.86	1.016	0.010	0.852
<i>Total FA (IMF)</i>	100.00	100.00	100.00	n/a	n/a	n/a

^aValues were reported as least squares means.

^b*P* values were obtained from the orthogonal polynomial contrast test.

^cCommon name of FA: C10:0 (capric acid), C12:0 (lauric acid), C14:0 (myristic acid), C15:0 (pentadecanoic acid), C16:0 (palmitic acid), C17:0 (heptadecanoic acid), C18:0 (stearic acid), C20:0 (arachidic acid), C14:1 n-5 (myristoleic acid), C16:1 n-7 (palmitoleic acid), C17:1 n-8 (heptadecenoic acid), C18:1 trans (eliadic acid), C18:1 n-9 (oleic acid), C20:1 n-9 (gadoleic acid), C24:1 n-9 (nervonic acid), C18:2 n-6 (linoleic acid), C20:2

434 (eicosadienoic acid), C18:3 n-3 (α -linolenic acid), C20:3 n-6 (γ -linolenic acid), and C20:4
435 n-6 (arachidonic acid).

436 ^dSFA = C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0; MUFA =
437 C14:1 n-5 + C16:1 n-7 + C17:1 n-8 + C18:1 trans + C18:1 n-9 + C20:1 n-9 + C24:1 n-9;
438 PUFA = C18:2 n-6 + C20:2 + C18:3 n-3 + C20:3 n-6 + C20:4 n-6.

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Figure Captions

Figure 1. The fatty acid saturation index [SI; SFA/(MUFA + PUFA)] (**A**) and the stearoyl-CoA desaturase (SCD) activity [(C16:1 n-7 + C18:1 n-9)/(C16:0 + C16:1 n-7 + C18:0 + C18:1 n-9)] (**B**) of the intramuscular fat (IMF) in *longissimus dorsi* of late-stage finishing pigs fed a lysine-deficient diet (Diet 1), a lysine-adequate diet (Diet 2), and a lysine-excess diet (Diet 3). While the error bars denote SD, the means without a common letter (i.e., a or b) differ ($P < 0.05$).

(Figure 1 for “CJAS-2017-0083.R1”)