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Original Research Article

Comparison of the meat quality and fatty acid profile of muscles in finishing Xiangcun Black pigs fed varied dietary energy levels

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ABSTRACT

To study the effects of dietary energy level on the meat quality of different muscles in finishing pigs, 400 Xiangcun Black pigs (BW = 79.55 ± 4.77 kg) were randomly assigned to 5 treatments with varied calculated digestive energy (DE) at 3,050, 3,100, 3,150, 3,200 and 3,250 kcal/kg, respectively. Each treatment had 8 replicates with 10 pigs per replicate. Meat quality, amino acid and fatty acid composition were tested in this study. No differences in average daily gain, average daily feed intake or feed-to-gain ratio ($P > 0.05$) were observed among dietary treatments. Glycogen concentrations of longissimus dorsi (LD) muscle in DE3150 was higher than those in other groups ($P < 0.05$). The crude fat concentration of biceps femoris (BF) muscle in DE3250 tended to be higher than that in DE3150 and DE3100 groups ($P < 0.05$). Pigs in DE3250 and DE3200 had higher fiber density and smaller cross-sectional area of BF muscle than those in DE3150 ($P < 0.05$). Pigs in DE3150 had the highest Cu concentration in LD muscle compared with those in DE3200, DE3250 ($P < 0.05$). The C16:1 proportion of LD muscle was lower ($P < 0.01$) and C20:1 was higher ($P < 0.05$) in DE3050 than that in the other dietary treatments. The C18:3n6 and C20:3n6 proportions of BF muscle in DE3150 were higher than those in DE 3050, DE3200 and DE3250 ($P < 0.05$). For LD muscle, mRNA expressions of type *I* and *IIa MyHC* in group DE3150 were higher than other treatments ($P < 0.01$). The LD muscle in DE3150 expressed higher *PPARd* than in other groups ($P < 0.01$). Pigs in DE3100 expressed higher *FOX1* than in DE3200 and DE3250 ($P < 0.05$). Sterol-regulatory element binding proteins (*SREBPa*) mRNA expression decreased linearly when dietary energy level increased in BF muscle ($P < 0.01$). In conclusion, a 200 kcal/kg decrease in digestible energy for 4 consecutive weeks did not affect growth performance of Xiangcun Black pigs. Furthermore, LD and BF muscle respond differently to dietary energy level, and meat quality was improved by the medium energy level during the finishing phase.

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1. Introduction

Meat quality is of great importance to the consumer because they prefer pork with a higher quality in tenderness, juiciness, and flavor (Henchion et al., 2014). Intramuscular fat (IMF) content and fatty acid composition are the main factors affecting meat quality and strongly depend on the diet (Wood et al., 2008). Restriction during the finishing phase reduces IMF content (Razmaitė et al., 2021). Moreover, fatty acid composition are amenable to change

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when using different diets and feeding regimens for pigs (Wood et al., 2008). Inadequate supply of dietary energy lowers concentrations of saturated and monounsaturated fatty acids in their overall profile (Daza et al., 2007). Local pigs are important resources for improving meat quality because of their greater ability to deposit fat in muscle (Gan et al., 2020; Huang et al., 2020). Xiangcun Black pigs, which are the offspring of Taoyuan black × Duroc, are different from Duroc, Yorkshire, and Landrace pigs, as they usually possess a good adaptation to environmental conditions and more frequently demonstrate higher meat quality (Poklukar et al., 2020). Whether Xiangcun Black pigs can be fed low energy diets without affecting meat quality is still not well understood.

Skeletal muscle accounts for approximately 50% of body mass in mammals. Skeletal muscle is composed of various muscle fibers that exhibit different properties, such as glycolysis, oxidative metabolism, and contraction (Gundersen, 2011). Differences in skeletal muscle fiber types directly affect meat quality postmortem which include pH, meat color, and drip loss (Ryu and Kim, 2005). Transformation of skeletal muscle fiber types was accompanied by a change of fatty acid composition (Joo et al., 2017). Longissimus dorsi (LD) and biceps femoris (BF) muscles represent the most important pork cuts, the loin and ham. Differences in gene expression were observed between LD and BF muscles, where LD muscle seems to have more active muscular and cell growth, while BF has a more active lipid metabolism and fat deposition (Ayuso et al., 2016).

In this study, we performed a comparative analysis of the effect of dietary energy level on BF (fast muscle or white muscle) and LD (slow muscle or red muscle) muscle. The goal was to evaluate the effect of dietary energy level on meat quality, myofibrillar morphology, mineral element, amino acid, fatty acid composition of different muscles in Xiangcun Black pigs.

2. Material and methods

2.1. Animal ethic statement

The experimental design and procedures in this study were reviewed and approved by the Animal Care and Use Committee of the Institute of Subtropical Agriculture, Chinese Academy of Science under ethic approval number ISA-2016-058.

2.2. Animals and experimental treatments

A total of 400 Xiangcun Black pigs (castrated male-to-female ratio = 1:1, initial body weight = 79.55 ± 4.77 kg) were randomly assigned to 5 treatments. Each treatment had 8 replicates, with 10 pigs per replicate. Pigs were fed a corn-soybean meal-based diet that met the NRC (2012) requirements for growing-finishing pigs, but with different digestive energy (DE) levels, 3,050, 3,100, 3,150, 3,200 and 3,250 kcal/kg diet, and named as DE3050, DE3100, DE3150, DE3200, and DE3250, respectively (Table 1). Pigs were raised in a pig farm under commercial conditions, with ad libitum access to feed and water. Feed intake was recorded every day. Body weight was measured at the beginning and end of the experiment. The experiment was performed between June and July 2016 and lasted 30 days. Housing conditions were controlled by a natural ventilation system.

2.3. Sample collection

At the end of the feeding trial, animals were transported to modern slaughterhouses of Ziyuan group (Changsha, Hunan, China). After arrival, pigs were allowed to rest for 4 h. Animals with medium body weight were slaughtered via electrical stunning followed by exsanguination. Carcasses were dehaired via scalding, eviscerated, and split vertically down the midline. Dressing percentage was calculated as hot carcass weight/live body weight × 100%. Midline backfat depths were measured opposite the first rib, the 6th rib and last rib from the right half of the carcass. The right carcass at 10th rib was used to measure loin-eye area (height × width × 0.7 cm^2), meat color and marbling score (scale from 1 to 3, subjectively evaluated, with 0 referring to “devoid” and 3 referring “overly abundant”). Muscles LD and BF were collected and rapidly frozen in liquid nitrogen and then stored at −80 °C until analysis. Tissue samples of LD and BF were fixed 4% polyformaldehyde until analysis.

2.4. Meat quality measurements

Meat color, including lightness (L*), redness (a*) and yellowness (b*), were measured at 45 min and 24 h postmortem on LD muscle using a hand-held colorimeter (CR-410, Kinica Minolta Sensing Inc., Osaka, Japan). The pH value was measured at 45 min and 24 h postmortem by inserting a pH probe (Matthaus pH Star, Germany) into the left LD muscle between the 10th and 11th rib. Drip loss of LD muscle in percent was determined as follows; approximately 50 g of sample from the 10th rib chop on the left side of the carcass was weighed and suspended on a barbless hook. The hook passed through a small hole on the bottom of an inverted plastic cup, placed inside of a Whirl-Pak bag, suspended for 12 or 24 h at 4 °C before being removed from the hook, and reweighed. For cooking loss determination, approximately 100 g of LD muscle was weighed and cooked for 30 min to an internal temperature of 70 °C. Afterwards, the cooked samples were cooled to room temperature and reweighed. Cooking loss was calculated as percentage loss during cooking.

Table 1
Calculated ingredient composition of finishing diets (%, as-fed basis).[1]

Item	DE3050	DE3100	DE3150	DE3200	DE3250
Ingredients					
Corn	58.62	62.02	65.42	68.81	72.25
Soya bean meal (43% CP)	17.30	18.26	19.22	20.17	21.16
Wheat bran	21.10	16.72	12.36	8.01	3.54
Limestone	1.30	1.29	1.27	1.26	1.19
Premix[2]	0.50	0.50	0.50	0.50	0.50
Choline Chloride	0.08	0.08	0.08	0.08	0.08
Dicalcium phosphate	0.49	0.52	0.55	0.57	0.69
Salt	0.45	0.46	0.46	0.47	0.48
L-Lysine HCl	0.16	0.15	0.14	0.13	0.11
Total	100.00	100.00	100.00	100.00	100.00
Calculated values					
CP	15.5	15.5	15.5	15.5	15.5
Ca	0.65	0.65	0.65	0.65	0.65
P	0.54	0.52	0.50	0.48	0.47
Digestible P	0.23	0.23	0.23	0.23	0.24
Salt	0.50	0.50	0.50	0.50	0.50
Lys[3]	0.84	0.84	0.84	0.84	0.84
DE, kcal/kg	3050	3100	3150	3200	3250
Crude fiber	3.70	3.40	3.10	2.90	2.60
Crude fat	3.20	3.20	3.10	3.10	3.10

[1] DE3050, DE3100, DE3150, DE3200, DE3250 represent diets with digestive energy 3,050, 3,100, 3,150, 3,200, 3,250 kcal/kg respectively.
[2] Provided the following quantities of vitamins and micro-minerals per kilogram of complete diet: vitamin A as retinyl acetate, 1,300 IU; vitamin D_3 as cholecalciferol, 150 IU; vitamin E as DL-alpha tocopheryl acetate, 11 IU; vitamin K as menadione dimethylpyrimidinol bisulfite, 0.5 mg; thiamin as thiamine mononitrate, 1.0 mg; riboflavin, 2.0 mg; pyridoxine as pyridoxine hydrochloride, 1.0 mg; vitamin B_{12}, 6.0 mg; D-pantothenic acid as D-calcium pantothenate, 7.0 mg; niacin, 7.5 mg; folic acid, 0.3 mg; biotin, 0.05 mg; Cu, 3.5 mg as copper sulfate; Fe, 50 mg as ferrous sulfate; I, 0.14 mg as ethylenediamine dihydride; Mn, 2.0 mg as manganese sulfate; Se, 0.25 mg as sodium selenite; and Zn, 50 mg as zinc sulfate.
[3] Amino acids are indicated as standardized ileal digestible AA.

2.5. Nutrient measurement

Muscles were freeze-dried for 72 h. Crude fat content of freeze-dried muscle was extracted by Soxhlet extraction using aether petrolei as solvent (AOAC, 1990). Kjeldahl method was used to determine the crude protein content of freeze-dried muscle. Glycogen content was tested in fresh meat sample using a commercial kit (Nanjing Jiancheng Institute of Bioengineering, China) according to manufacturer's instructions.

2.6. Myofibrillar morphology

The cross-sectional area of myofibers in muscle were measured by classic hematoxylin and eosin staining. Briefly, muscles from the polyformaldehyde were washed in running water overnight. They were treated with increasing concentrations of acetone, rinsed, and embedded in solid paraffin. Slides were obtained by cutting muscles wrapped in the paraffin, and then stained by hematoxylin and eosin. Images of the slides were captured with Leica RM2135 (Leica Microsystems, Wetzlar, Germany) inverted microscope and Cannon camera. Fiber density, cross-sectional area and fiber diameter was analyzed from 100 fibers per pig using Image-Pro Plus software (Media Cybernetics Inc., Silver Spring, MD).

2.7. Mineral element analysis

The mineral element content of freeze-dried LD muscle was determined by inductively coupled plasma optical emission spectrometry (ICP-OES; ICP 720 ES; Agilent, USA). Approximately 5 g samples were weighed in triplicate and digested in a mixture of nitric and perchloric acids at 180 °C for 2 h. Samples were dried at 260 °C and redissolved in 5 mL of 1% HNO_3. Samples were diluted with 1% HNO_3, filtered and subjected to mineral element analyses. The quantity of mineral elements was determined by comparing the peak areas of their standard.

2.8. Amino acid analysis

Amino acid contents of freeze-dried muscles was analyzed via an Amino Acid Analyzer (L-8900, HITACHI, Japan). Approximately 0.50-g dried muscle was hydrolyzed in a sealed glass tube with 10 mL 6 mol/L HCl at 105 °C for 24 h. The hydrolysates were diluted with double-distilled water. One milliliter of diluted hydrolysate was dried via a vacuum drier, and then redissolved in 1 mL of 0.02 mol/L HCl, filtered through a 0.22-μm membrane, and used to measure AA concentration. Identity and quantity of AA were determined by comparing to the retention times and peak areas of their standard.

2.9. Fatty acid analysis

Fatty acid content of freeze-dried muscles was determined in an Agilent 7890A gas chromatographer equipped with SP-2560 column (100 m × 250 μm × 0.2 μm) (Agilent Technologies Inc., Palo Alto, CA) using a method provided previously (Martin et al., 2008). Fatty acid was identified by matching their retention times with authentic standards (Sigma Chemicals, St. Louis, MO). Concentrations of fatty acid were quantified according to their peak area and expressed as percentage of total fatty acids.

2.10. Gene expression analysis

Total RNA was isolated from muscle using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Then CDNA was obtained using 1.0 μg of total RNA treated with DNase I (Fermentas Inc., Glen Burnie, MD) and then using First-Strand cDNA Synthesis Kit (Fermentas Inc.). Primers were designed with online Primer-blast in NCBI (Supplementary Table). The RT-PCR was performed on an ABI 7900HT RT-PCR system (Applied Biosystems, Branchburg, NJ). The mRNA expression levels of genes related to lipid metabolism and myosin heavy chain were calculated by $2^{-\triangle\triangle Ct}$ method described previously (Livak and Schmittgen, 2001).

2.11. Statistical analysis

Data were analyzed by a one-factor general linear model (GLM) using the SAS 8.2 software package (SAS Inst. Inc., Cary, NC). Duncan's multiple range test was used to indicate the significance of differences at $P < 0.05$. Linear, quadratic, and cubic contrasts were analyzed using regression analysis (REG) procedure of SAS to assess the significance of the relevance. Data were expressed as least-squares means ± SEM. Means were considered to be significantly different when $P < 0.05$ and a tendency when $0.05 \leq P \leq 0.10$.

3. Results

3.1. Growth performance

There were no differences in ADG, ADFI or F:G ($P > 0.05$) among different groups with varied energy levels in finishing pigs (Table 2).

3.2. Carcass traits and meat quality

Dietary energy levels did not affect dressing percentage, loin-eye area or backfat thickness of finishing pigs ($P > 0.05$) (Table 3). The pH values at 45 min and 24 h, L*, a*, b* value at 45 min and 24 h postmortem, cooking loss, drip loss and marble score were not affected by treatments ($P > 0.05$). Crude protein and crude fat concentration of LD muscle were not affected by dietary energy levels ($P > 0.05$). Glycogen concentration of LD muscle in group DE3150 was higher than those in the other treatments ($P < 0.05$), and it tended to decrease in a cubic manner with dietary energy level ($P = 0.06$). Glycogen and crude protein content of BF muscle were not affected by dietary treatments ($P > 0.05$). Compared with DE3250, pigs in groups DE3100 and DE3150 tended to have lower crude fat concentrations in BF muscle ($P = 0.06$), with no significant difference between groups DE3250, DE3050 and DE3200 ($P > 0.05$). Crude fat of BF muscle failed to reach a significant level when correlated with dietary energy level.

3.3. Myofibrillar morphology

Dietary energy levels did not affect fiber diameter, cross-sectional area or density of LD muscle ($P > 0.05$) (Table 4). Muscle BF in DE3150 had larger fiber diameter and fiber cross-sectional area than that in DE3250 and DE3200 groups ($P < 0.05$), with no significant difference compared with DE3100 ($P > 0.05$). Among all the treatments, pigs in DE3250 had the highest BF muscle fiber density, followed by DE 3200 ($P < 0.01$), which in turn were greater than that in groups DE3050, DE3100 and DE3150 ($P < 0.01$). Density of BF muscle increased ($P < 0.01$), but cross-sectional area tended to decrease ($P = 0.098$) linearly with the increasing dietary energy levels.

3.4. Mineral element content

There were no significant differences between diets with different energy levels in regards to Fe, Mg, Cr, Mn, Zn, P and Ca concentrations of LD muscle in finishing pigs ($P > 0.05$) (Table 5).

Table 2
Effect of dietary energy level on growth performance of finishing pigs.[1]

Item	DE3050	DE3100	DE3150	DE3200	DE3250	SEM	*P*-value
Initial BW, kg	79.71	79.89	80.01	79.95	78.22	0.79	0.945
Final BW, kg	91.49	91.20	92.17	91.95	88.04	1.03	0.711
ADFI, kg/d	2.70	2.57	2.60	2.60	2.42	0.05	0.516
ADG, g/d	569.4	580.5	622.3	616.2	570.0	18.13	0.807
F:G, g/g	4.80	4.51	4.26	4.25	4.29	0.08	0.150

BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; F:G = the ratio of feed to body weight gain.
[1] DE3050, DE3100, DE3150, DE3200, DE3250 represent diets with digestive energy 3,050, 3,100, 3,150, 3,200, 3,250 kcal/kg respectively. And, no variable met the 0.15 significance level for entry into the model when regression analysis (REG) procedure run.

Table 3
Effect of dietary energy level on carcass performance and meat quality of finishing pigs.[1]

Item	DE3050	DE3100	DE3150	DE3200	DE3250	SEM	*P*-value	P_{linear}	$P_{quadratic}$	P_{cubic}
Carcass performance										
Body weight, kg	94.75	100.69	101.69	95.81	96.00	1.02	0.139	NS	NS	NS
Dressing percentage, %	69.90	68.93	66.58	69.37	69.87	0.89	0.749	NS	NS	NS
Loin-eye area, cm^2	20.70	23.73	24.28	25.11	23.54	0.77	0.462	NS	NS	NS
Backfat thickness, mm	37.17	38.44	35.19	36.39	38.99	1.03	0.775	NS	NS	NS
Meat quality										
$pH_{45\ min}$	5.78	5.75	6.06	5.77	5.66	0.07	0.472	NS	NS	NS
$pH_{24\ h}$	5.62	5.69	5.59	5.56	5.54	0.04	0.753	NS	NS	NS
$L^*_{45\ min}$	54.99	53.75	52.35	52.09	55.41	0.58	0.269	NS	NS	NS
$a^*_{45\ min}$	15.40	14.74	14.49	15.48	14.94	0.22	0.551	NS	NS	NS
$b^*_{45\ min}$	5.30	5.23	4.65	4.90	5.29	0.14	0.492	NS	NS	NS
$L^*_{24\ h}$	56.15	53.88	55.78	55.68	57.06	0.75	0.755	NS	NS	NS
$a^*_{24\ h}$	14.48	13.80	13.80	14.29	14.60	0.26	0.785	NS	NS	NS
$b^*_{24\ h}$	6.81	6.90	7.01	7.02	7.51	0.21	0.844	NS	NS	NS
Cooking loss, %	43.78	41.63	42.57	42.49	42.50	0.41	0.594	NS	NS	NS
Drip loss at 12 h, %	2.19	2.13	1.67	0.98	2.31	0.28	0.542	NS	NS	NS
Drip loss at 24 h, %	3.33	3.76	3.10	3.77	4.00	0.37	0.936	NS	NS	NS
Marble score	2.13	2.50	1.75	2.25	2.25	0.13	0.475	NS	NS	NS
Nutrient content										
Muscle LD[2]										
Crude protein, %	16.26	16.53	19.58	16.50	18.25	0.88	0.706	NS	NS	NS
IMF, %	3.13	2.83	2.98	2.91	3.53	0.24	0.895	NS	NS	NS
Glycogen, mg/g	4.08^B	4.27^B	5.48^A	4.26^B	3.42^B	0.17	0.012	NS	−0.137	−0.063
Muscle BF[2]										
Crude protein, %	17.28	17.80	19.05	16.60	18.74	0.43	0.372	NS	NS	NS
IMF, %	3.95	3.29	2.73	3.74	5.42	0.30	0.086	+0.134	+0.129	+0.123
Glycogen, mg/g	3.96	3.56	4.44	3.69	3.55	0.17	0.453	NS	NS	NS

IMF = intramuscular fat; LD = longissimus dorsi; BF = biceps femoris.
[A, B] Mean values within a row without a common upper case superscript letter differ in $P < 0.05$.
[1] DE3050, DE3100, DE3150, DE3200, DE3250 represent diet with digestive energy 3,050, 3,100, 3,150, 3,200, 3,250 kcal/kg respectively. NS means that no variable met the 0.15 significance level for entry into the model when regression analysis (REG) procedure run; + means positive effect; − means negative effect.
[2] Crude protein and IMF concentrations were given by fresh based meat sample; glycogen was tested by fresh meat sample.

Pigs in DE3250 had lower Cu concentration in LD muscle compared with DE3050 and DE3150 ($P < 0.05$), with no significant differences when compared with DE 3100 and DE3200 ($P > 0.05$). The S concentration of LD muscle in DE3100 was significantly greater than those in groups DE3050 and DE3150 ($P < 0.05$), with no significant difference compared with DE3200 and DE3250 ($P > 0.05$). In muscle LD, Cu and Mn decreased linearly ($P = 0.040$ and 0.008, respectively) and Ca concentration tended to decrease ($P = 0.060$) with the increasing dietary energy levels.

3.5. Amino acid content

The dietary energy level did not affect aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cysteine (Cys), valine (Val), methionine, isoleucine (Ile), leucine, tyrosine, phenylalanine, lysine, NH_3, histidine (His), arginine, proline of LD and BF muscle ($P > 0.05$) (Table 6). Contents of essential amino acids, non-essential amino acids, umami AA, sweet AA and bitter AA were not affected by dietary treatment ($P > 0.05$). For LD muscle, Cys concentration increased linearly ($P = 0.040$) and Val, Ile, His concentrations tended to increase ($P = 0.099$, 0.087, 0.080, respectively) with the dietary energy level. Other AA related to non-flavor amino acids tended to increase quadratically with increasing dietary energy level ($P = 0.073$).

3.6. Fatty acid content

The C14:0 proportion of LD muscle in DE3150 was higher than those in groups DE3050 and DE3200 ($P < 0.05$), with no significant difference when compared with DE3100 and DE3250 (Table 7). The proportion of C16:1 of LD muscle was lower but C20:1 was higher in DE3050 than those in other groups ($P < 0.01$). The DE3050 was also highest in C17:0 in LD muscle, higher than that in DE3200 and DE3250 ($P < 0.05$), but with no significant difference compared with DE3100 and DE3150. Concentrations of saturated fatty acids (SFA), monounsaturated fatty acid (MFA), polyunsaturated fatty acid (PUFA), n6 or n3 PUFA were not affected by treatment ($P > 0.05$). Fatty acid C17:0 and C20:1 of LD muscle decreased linearly ($P = 0.006$) and C20:0 tended to decrease ($P = 0.058$) with the increasing dietary energy levels.

For fatty acid concentration of BF muscle, DE3100 and DE3250 had higher C16:1 concentrations than in DE3050 ($P < 0.05$), but

Table 4
Effects of dietary energy level on myofibrillar morphology of finishing pigs.[1]

Item	DE3050	DE3100	DE3150	DE3200	DE3250	SEM	*P*-value	P_{linear}	$P_{quadratic}$	P_{cubic}
Muscle LD										
Diameter, μm	19.34	20.53	19.23	20.41	20.24	0.36	0.677	NS	NS	NS
Cross-sectional area, μm^2	2,714.30	3,026.10	2,626.10	2,965.70	2,904.30	98.81	0.670	NS	NS	NS
Density, N/mm^2	226.71	228.88	245.61	231.90	232.74	6.18	0.886	NS	NS	NS
Muscle BF										
Diameter, μm	13.85^{ABC}	14.84^{AB}	15.27^{A}	13.10^{C}	13.56^{BC}	0.40	0.026	NS	NS	NS
Cross-sectional area, μm^2	$1,696.9^{B}$	$2,029.5^{AB}$	$2,098.4^{A}$	$1,521.2^{B}$	$1,580.8^{B}$	104.83	0.008	−0.0977	0.094	0.090
Density, N/mm^2	214.43^{c}	223.81^{c}	225.97^{c}	279.64^{b}	360.08^{a}	11.03	<0.0001	<0.0001	<0.0001	<0.0001

LD = longissimus dorsi; BF = biceps femoris.
[A, B, C] Mean values within a row with different superscripts differ significantly at $P < 0.05$.
[a, b, c] Mean values within a row with different superscripts differ significantly at $P < 0.01$.
[1] DE3050, DE3100, DE3150, DE3200, DE3250 represent diet with digestive energy 3,050, 3,100, 3,150, 3,200, 3,250 kcal/kg respectively. NS means that no variable met the 0.15 significance level for entry into the model when regression analysis (REG) procedure run; + means positive effect; − means negative effect.

Table 5
Effects of dietary energy level on micronutrient content of longissimus dorsi muscle in finishing pigs, freeze-dried base.[1]

Item	DE3050	DE3100	DE3150	DE3200	DE3250	SEM	*P*-value	P_{linear}	$P_{quadratic}$	P_{cubic}
Fe, μg/g	1,400.5	1,167.8	1,673.3	1,278.9	1,352.7	131.29	0.798	NS	NS	NS
Mg, μg/g	2,395.8	2,347.4	2,469.1	2,505.9	2,396.6	47.56	0.838	NS	NS	NS
Cr, μg/g	16.40	20.34	18.97	16.58	13.97	0.99	0.339	NS	NS	NS
Cu, μg/g	21.82^{AB}	18.19^{BC}	22.54^{A}	18.56^{BC}	17.24^{C}	0.57	0.020	−0.037	−0.036	−0.036
Mn, μg/g	19.69	17.30	14.60	13.07	12.09	1.01	0.135	−0.008	−0.008	−0.008
Zn, μg/g	246.94	237.09	252.61	256.35	219.58	6.75	0.445	NS	NS	NS
P, mg/g	17.69	17.80	17.66	19.09	18.28	0.39	0.744	NS	NS	NS
Ca, mg/g	3.82	3.73	3.77	3.40	3.18	0.12	0.388	−0.058	−0.057	−0.057
S, mg/g	14.88^{B}	22.21^{A}	15.49^{B}	19.57^{AB}	18.73^{AB}	0.80	0.037	NS	NS	NS

[A, B, C] Mean values within a row with different superscripts differ significantly at $P < 0.05$.
[1] DE3050, DE3100, DE3150, DE3200, DE3250 represent diet with digestive energy 3,050, 3,100, 3,150, 3,200, 3,250 kcal/kg respectively. NS means that no variable met the 0.15 significance level for entry into the model when regression analysis (REG) procedure run; + means positive effect; − means negative effect.

with no significant differences compared with DE3150 and DE3200 ($P > 0.05$). The C18:3n6 and C20:3n6 concentration in DE3150 was higher than that in groups DE3050, DE3200 and DE3250 ($P < 0.05$), but the difference between DE3150 and DE3100 was not significant ($P = 0.056$). The C14:0 of BF muscle increased linearly ($P < 0.05$) and C16:1 ($P = 0.067$) tended to increase linearly with increasing dietary energy level. But C17:0 tended to decrease linearly with increasing dietary energy level ($P = 0.053$). The PUFA, n6 PUFA and n6:n3 values tended to decrease in a cubic manner with dietary energy level ($P = 0.082$, 0.076, 0.087, respectively).

3.7. Gene expression

Expression of genes related to lipid metabolism in LD muscle are shown in Table 8. Expressions of type *I* and type *IIa MyHC* in DE3150 were higher than that in other groups ($P < 0.01$). The mRNA expression of type *IIx MyHC* were higher in DE3100 than in DE3050 and DE3150 ($P < 0.05$), but with no significant difference when compared with DE3200 or DE3250 ($P > 0.05$). Fatty acid synthase (*FAS*) expression was higher in DE3100 than in groups DE3150, DE3200 and DE3250 ($P < 0.05$), but with no significant difference compared with DE3050 ($P > 0.05$). The LD muscle in DE3150 expressed higher uncoupling protein 3 (*UCP3*) and *PPARd* than in other groups ($P < 0.01$). Expression of CCAAT/enhancer binding protein alpha (*CEBPa*) was lower in DE3250 than that in other groups ($P < 0.01$). Pigs in DE3200 and DE3250 had a lower expression of silent mating type information regulation 2 homolog (*Sirt*) compared with the other three groups ($P < 0.01$). Other genes' expression such as type *IIb MyHC*, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*PGC-1α*), *UCP2* and hormone-sensitive triglyceride lipase (*HSL*) in LD muscle were not affected by dietary energy level. Expression of *FOX1, UCP3, PPARd* decreased and *Sirt* and *CEBPa* increased ($P < 0.01$) linearly with dietary energy level in LD muscle.

For BF muscle, expression of type *IIx, IIa, IIb MyHC, PGCa, FAS, HSL, Sirt* and *CEBPa* were not affected by treatment ($P > 0.05$). Type *I MyHC* was higher in DE3100 than in other groups ($P < 0.05$). Expression of *UCP2* and acetyl CoA carboxylase (*ACC*) was higher in DE3100 than in other groups ($P < 0.05$). Pigs in DE3100 also had a higher expression of lipoprteinlipase (*LPL*) gene than other groups ($P < 0.05$), except DE3250. Adenosine 5′-monophosphate (AMP)-activated protein kinase (*AMPK*) expression was lower in DE3200 and DE3250 than that in DE3050 ($P < 0.05$), but with no significant difference when compared with DE3100 and DE3150 ($P > 0.05$). The *SREBP* expression was higher in DE3050 than in other groups ($P < 0.05$). Pigs in group DE3100 expressed higher *FOX1* than in DE3200 and DE3250 ($P < 0.05$), but with no significant difference compared with DE3050 and DE3150 ($P > 0.05$). Only *SREBP* expression decreased linearly with dietary energy level in BF muscle ($P < 0.01$).

4. Discussion

Dietary energy density did not affect growth performance during finishing period, which was similar to other reports. A 0.42-MJ decrease in NE (Kerr et al., 2003) or 1.26 MJ decrease in ME (Kil et al., 2013) in finishing pig diets did not significantly affect growth performance. Our experiment only lasted 4 weeks. However, the G:F ratio decreased when dietary ME level decreased from 13.82 to 13.40 MJ/kg during a successive 12 weeks study (Fang et al., 2019).

There was no significant difference in crude protein or crude fat of LD muscle when pigs were fed diets varying in DE (3,050 to 3,250 kcal/kg). Other studies have reported similar results (100 or 511 kcal/kg decreased) (Kil et al., 2011; Fang et al., 2019). No

Table 6
Effects of dietary energy level on amino acid composition of muscles in finishing pigs (μg/100 mg).[1]

Item	DE3050	DE3100	DE3150	DE3200	DE3250	SEM	*P*-value	P_{linear}	$P_{quadratic}$	P_{cubic}
Muscle LD										
Lys	5.99	6.17	6.48	6.81	6.90	0.18	0.534	+0.102	+0.099	+0.109
Ile	3.19	3.29	3.42	3.65	3.70	0.09	0.442	+0.087	+0.078	+0.083
Leu	5.46	5.62	5.86	6.21	6.26	0.16	0.533	+0.113	+0.106	+0.115
Val	3.48	3.56	3.64	3.95	3.98	0.10	0.438	+0.099	+0.082	+0.084
Thr	1.58	1.19	0.00	1.21	0.89	0.23	0.250	NS	NS	NS
Phe	2.78	2.84	2.93	3.14	3.17	0.08	0.528	+0.117	+0.103	+0.108
Met	1.62	1.79	1.78	1.90	1.89	0.06	0.757	NS	NS	NS
Asp	6.43	6.38	6.70	6.99	7.03	0.17	0.633	+0.125	+0.127	+0.144
Ser	2.79	2.73	2.74	2.99	2.99	0.07	0.632	NS	NS	NS
Glu	10.99	10.73	10.79	11.66	11.70	0.29	0.708	NS	NS	NS
Gly	2.74	2.79	2.92	3.08	3.05	0.08	0.657	NS	NS	NS
Ala	3.80	3.87	3.97	4.25	4.25	0.11	0.599	NS	+0.139	+0.146
Cys	0.69	0.66	0.72	0.81	0.82	0.02	0.194	+0.040	+0.028	+0.028
Tyr	2.40	2.50	2.59	2.69	2.71	0.07	0.736	NS	NS	NS
NH_3	0.88	0.95	0.98	1.03	1.01	0.02	0.538	NS	NS	NS
His	3.12	3.15	3.35	3.61	3.57	0.09	0.422	+0.080	+0.079	+0.093
Arg	4.22	4.33	4.49	4.79	4.81	0.12	0.560	+0.127	+0.117	+0.126
Pro	2.95	2.95	2.93	3.23	3.23	0.08	0.603	NS	NS	NS
Total	66.89	65.48	66.28	72.01	71.96	0.00	0.611	NS	+0.148	+0.148
EAA[2]	25.13	24.45	24.11	26.87	26.79	0.67	0.543	NS	NS	NS
NEAA	41.76	41.03	42.17	45.14	45.17	1.10	0.638	NS	+0.141	+0.148
Umami AA[3]	17.36	17.10	17.48	18.66	18.73	0.45	0.681	NS	NS	NS
Sweet AA	20.42	19.69	19.04	21.57	21.30	0.54	0.531	NS	NS	NS
Bitter AA[4]	27.44	27.07	28.05	29.95	30.09	0.75	0.587	+0.118	+0.110	+0.119
Other AA	1.66	1.61	1.70	1.84	1.83	0.04	0.417	+0.079	+0.073	+0.083
Muscle BF										
Lys	6.32	6.63	7.16	6.72	6.01	0.18	0.441	NS	NS	NS
Ile	3.33	3.43	3.79	3.57	3.06	0.10	0.314	NS	NS	NS
Leu	5.83	6.12	0.64	6.21	5.55	0.16	0.413	NS	NS	NS
Val	3.70	3.84	4.22	3.95	3.45	0.11	0.344	NS	NS	NS
Thr	3.27	3.42	3.73	3.45	3.13	0.09	0.404	NS	NS	NS
Phe	3.00	3.14	3.44	3.21	2.86	0.08	0.386	NS	NS	NS
Met	1.57	1.52	1.53	1.72	1.39	0.08	0.872	NS	NS	NS
Asp	6.21	6.48	7.08	6.59	6.01	0.17	0.477	NS	NS	NS
Ser	3.08	3.29	3.60	3.25	2.96	0.09	0.370	NS	NS	NS
Glu	11.96	12.40	13.93	13.22	11.32	0.40	0.406	NS	NS	NS
Gly	2.93	2.98	3.69	3.03	2.79	0.13	0.347	NS	NS	NS
Ala	4.15	4.34	4.34	4.33	3.95	0.08	0.564	NS	NS	NS
Cys	0.64	0.82	0.82	0.81	0.67	0.03	0.329	NS	NS	NS
Tyr	2.54	2.68	2.77	2.63	2.44	0.07	0.736	NS	NS	NS
NH3	0.98	1.04	1.23	1.00	0.98	0.03	0.128	NS	NS	NS
His	2.78	2.94	3.14	3.01	2.62	0.08	0.380	NS	NS	NS
Arg	4.57	4.78	5.29	4.85	4.34	0.14	0.442	NS	NS	NS
Pro	3.50	3.69	4.40	3.60	3.34	0.13	0.190	NS	NS	NS
Total	70.34	73.53	80.80	75.15	66.86	0.00	0.407	NS	NS	NS
EAA[2]	27.01	28.09	30.52	28.83	25.45	0.77	0.438	NS	NS	NS
NEAA	43.34	45.44	50.29	46.32	41.42	1.28	0.389	NS	NS	NS
Umami AA[3]	18.17	18.88	21.01	19.81	17.33	0.56	0.424	NS	NS	NS
Sweet AA[4]	23.25	24.34	26.93	24.38	22.18	0.66	0.364	NS	NS	NS
Bitter AA[5]	27.31	28.45	30.82	29.15	25.70	0.79	0.454	NS	NS	NS
Other AA	1.62	1.86	2.04	1.81	1.65	0.05	0.177	NS	NS	NS

LD = longissimus dorsi; BF = biceps femoris; EAA = essential amino acids; NEAA = non-essential amino acids.
[1] DE3050, DE3100, DE3150, DE3200, DE3250 represent diet with digestive energy 3,050, 3,100, 3,150, 3,200, 3,250 kcal/kg respectively. NS means that no variable met the 0.15 significance level for entry into the model when regression analysis (REG) procedure run; + means positive effect; − means negative effect.
[2] Essential amino acids include Lys, Ile, Leu, Val, Thr, Phe, Met.
[3] Umami AA include Glu, Asp.
[4] Sweet AA include Gly, Ala, Ser, Thr, Pro, Gln, Lys.
[5] Bitter AA include Tyr, Arg, His, Val, Met, Ile, Leu, Trp, Phe.

detrimental effect on cooking loss, drip loss or meat color of LD muscle was observed when DE of finishing pigs decreased from 3,250 to 3,050 kcal/kg. Meat quality of LD muscle did not depend on the level of nutrition (Więcek et al., 2011). Other reports also showed that cooking loss was not affected by dietary ME (14.65 to 14.24 MJ/kg) (Meng et al., 2010), (3,398 vs 3,645 kcal/kg) (Widmer et al., 2008). Decreasing energy level in the diet did not affect meat color (Apple et al., 2004; Fang et al., 2019). Longissimus dorsi muscle is a glycolytic muscle, containing 51% of type *IIb MyHC* (Toniolo et al., 2004). Muscle LD in the medium energy diet (DE3150) had higher glucogen accompanied with a higher proportion of type *I* and *IIa MyHC* compared with high or low energy diets in the present study. It seems that under medium energy levels, skeletal muscle fiber type IIb transformed to type IIa and I fibers. Oxidative type I fibers are mitochondria-rich, preferring utilizing oxidative phosphorylation for energy production, while the glycolytic type II fibers depend on glycolytic metabolism to generate ATP (Barnard et al., 1971). The oxidative capacity of muscle in DE3150 increased as indicated by high *UCP3* expression. This more oxidative phenotype maybe regulated by *PPARd*, which is regulator of genes involved in type II to type I fiber transition, mitochondrial biogenesis and oxidative metabolic pathways

Table 7
Effects of dietary energy level on fatty acid composition of the muscle in finishing pigs (% of total fatty acids).[1]

Item	DE3050	DE3100	DE3150	DE3200	DE3250	SEM	*P*-value	P_{linear}	$P_{quadratic}$	P_{cubic}
Muscle LD										
C14:0	1.58^{B}	1.71^{AB}	1.82^{A}	1.51^{B}	1.67^{AB}	0.03	0.033	NS	NS	NS
C16:0	28.10	28.12	28.55	28.38	28.46	0.26	0.976	NS	NS	NS
C16:1	2.98^{B}	4.38^{A}	3.90^{A}	3.96^{A}	3.89^{A}	0.11	0.004	+0.114	NS	NS
C17:0	0.35^{A}	0.30^{AB}	0.29^{AB}	0.26^{B}	0.27^{B}	0.01	0.048	−0.006	−0.015	−0.030
C18:0	15.75	14.53	16.42	15.17	15.4	0.27	0.26	NS	NS	NS
C18:1n9t	0.20	0.26	0.17	0.20	0.23	0.01	0.056	NS	NS	NS
C18:1n9c	40.66	39.89	37.46	38.82	39.43	0.44	0.229	NS	NS	NS
C18:2n6c	7.04	7.11	7.38	7.82	7.23	0.31	0.936	NS	NS	NS
C20:0	0.29	0.24	0.26	0.25	0.25	0.01	0.065	−0.058	−0.112	NS
C18:3n6	0.03	0.04	0.04	0.05	0.04	0.00	0.606	NS	NS	NS
C20:1	1.13^{A}	0.98^{B}	1.01^{B}	0.97^{B}	0.93^{B}	0.02	0.016	−0.003	−0.008	−0.015
C18:3n3	0.41	0.42	0.43	0.46	0.44	0.02	0.886	NS	NS	NS
C20:3n6	0.21	0.28	0.28	0.25	0.23	0.01	0.402	NS	NS	NS
C20:4n6	1.20	1.62	1.97	1.66	1.40	0.09	0.082	NS	NS	NS
C22:6n3	0.09	0.13	0.15	0.23	0.14	0.02	0.428	NS	NS	NS
SFA	47.19	45.88	48.34	46.55	46.98	0.41	0.442	NS	NS	NS
MUFA	44.96	45.50	42.41	43.95	44.48	0.46	0.292	NS	NS	NS
PUFA	8.98	9.60	10.24	10.47	9.47	0.40	0.768	NS	NS	NS
MUFA/PUFA	5.47	4.88	4.51	4.35	5.10	0.22	0.527	NS	NS	NS
PUFA, n6	8.48	9.06	9.67	9.78	8.89	0.39	0.808	NS	NS	NS
PUFA, n3	0.50	0.55	0.58	0.69	0.58	0.03	0.319	+0.120	NS	NS
Ratio of n6:n3	18.17	18.09	17.65	14.64	16.23	0.83	0.622	NS	NS	NS
Muscle BF										
C14:0	1.57	1.60	1.51	1.62	1.76	0.03	0.052	+0.039	+0.016	+0.009
C16:0	25.97	26.04	24.88	26.73	26.73	0.31	0.314	NS	NS	NS
C16:1	3.03^{B}	4.01^{A}	3.51^{AB}	3.63^{AB}	3.94^{A}	0.10	0.037	+0.067	+0.103	+0.127
C17:0	0.43	0.36	0.36	0.37	0.28	0.02	0.288	−0.053	−0.053	−0.054
C18:0	15.50	14.47	14.31	14.89	14.33	0.28	0.621	NS	NS	NS
C18:1n9t	0.23	0.29	0.20	0.22	0.21	0.02	0.619	NS	NS	NS
C18:1n9c	35.18	34.48	35.08	35.47	36.40	0.41	0.681	NS	NS	NS
C18:2n6c	13.11	13.35	14.55	12.60	12.06	0.37	0.319	NS	NS	+0.145
C20:0	0.24	0.20	0.21	0.23	0.23	0.01	0.270	NS	NS	NS
C18:3n6	0.05^{B}	0.07^{AB}	0.09^{A}	0.06^{B}	0.06^{B}	0.00	0.014	NS	NS	NS
C20:1	1.02	0.94	1.03	1.00	0.98	0.02	0.767	NS	NS	NS
C18:3n3	0.71	0.68	0.68	0.67	0.73	0.02	0.898	NS	NS	NS
C20:3n6	0.39^{b}	0.43^{ab}	0.53^{a}	0.31^{b}	0.31^{b}	0.02	0.007	−0.103	−0.040	−0.022
C20:4n6	2.55	3.09	3.01	2.20	1.97	0.18	0.226	−0.124	−0.069	−0.052
C22:6n3	0.026	0.009	0.048	0.010	0.031	0.00	0.135	NS	NS	NS
SFA	44.73	43.60	42.31	44.85	44.29	0.49	0.489	NS	NS	NS
MUFA	39.46	39.72	39.82	40.31	41.53	0.48	0.678	NS	+0.132	+0.124
PUFA	16.83	17.62	18.90	15.84	15.16	0.53	0.222	NS	−0.116	−0.082
MUFA/PUFA	2.49	2.43	2.16	2.60	2.77	0.09	0.332	NS	NS	+0.128
PUFA, n6	16.09	16.93	18.17	15.17	14.40	0.52	0.212	NS	−0.110	−0.076
PUFA, n3	0.74	0.69	0.73	0.68	0.76	0.02	0.757	NS	NS	NS
Ratio of n6 to n3	21.91	25.50	26.30	22.91	19.32	1.00	0.197	NS	−0.143	−0.087

LD = longissimus dorsi; BF = biceps femoris; SFA = saturated fatty acids; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.
[A, B, C] Mean values within a row with different superscripts differ significantly at $P < 0.05$.
[a, b, c] Mean values within a row with different superscripts differ significantly at $P < 0.01$.
[1] DE3050, DE3100, DE3150, DE3200, DE3250 represent diet with digestive energy 3,050, 3,100, 3,150, 3,200, 3,250 kcal/kg respectively. NS means that no variable met the 0.15 significance level for entry into the model when regression analysis (REG) procedure run; + means positive effect; − means negative effect.

(Schuler et al., 2006). Obese individuals had a significantly lower percentage of type I and a higher percentage of type IIb/x muscle fibers than lean individuals (Gerrits et al., 2010). Increased amount of muscle glycogen available at the time of slaughter could contribute high values of ultimate pH. However, pH value of LD muscle was not affected by dietary energy level, which was similar to other reports (Apple et al., 2004; Widmer et al., 2008).

Compared with the high energy diet, the crude fat content of BF muscle, but not LD muscle, tended to be lower in the medium energy (DE3150) group. This was consistent with the report that only IMF in BF muscle but not LD muscle was affected by a pig's growth (Ayuso et al., 2016). Muscle BF showed enriched pathways involved in lipid metabolism and a more active lipid metabolism compared to LD muscle (Ayuso et al., 2016). The total number of fibers and the fiber density was related to IMF content (Kim et al., 2013). Group DE3150 had the lowest crude fat and had less fiber numbers per area than other groups. Intramuscular fat was lower in pigs with a 30% feed restriction compared to those fed ad libitum (Moore et al., 2017), but it was not affected by a moderate feed restriction of 20% (Batorek et al., 2012). In this study, dietary energy level greatly impacted markers of fatty acid synthesis, catabolism and oxidation in the muscles of pigs, as evidenced by the increased expression of *FAS* in LD muscle and increased expression of *LPL* and *ACC* in BF muscle of pigs fed lower energy (DE3100) diets. Genes *C/EBPa, PPARr* and *SREBP-1c* are key transcription regulators controlling expression of genes involved in lipogenesis and lipolysis (Schwenk et al., 2010). We noted that the high energy diet also induced a downregulation of genes *CEBPa* in LD muscle and *SREBPa* and *AMPK* in BF muscle, indicating that lipid synthesis may be increased in the high energy (DE3250) group. Intramuscular fat content (IMF) content may have a decisive influence on pig meat, where an increase in IMF may improve the eating quality of the meat (tenderness, juiciness and flavor) (Teye et al., 2006). Unfortunately, we did not test the meat quality of BF muscle.

Table 8
Effects of dietary energy level on genes expression of the muscle in finishing pigs.[1]

Genes	DE3050	DE3100	DE3150	DE3200	DE3250	SEM	*P*-value	P_{linear}	$P_{quadratic}$	P_{cubic}
Muscle										
I	6.31^{B}	10.78^{B}	21.01^{A}	5.48^{B}	11.63^{B}	1.06	0.002	NS	NS	NS
IIa	5.68^{B}	8.02^{B}	16.06^{A}	6.08^{B}	8.28^{B}	0.81	0.005	NS	NS	NS
IIx	6.55^{B}	14.23^{A}	11.62^{AB}	7.58^{B}	9.09^{AB}	0.70	0.046	NS	NS	NS
IIb	1.26	2.01	2.02	1.08	0.25	0.18	0.056	−0.080	−0.077	−0.073
PGCa	1.10	1.24	1.50	1.50	1.76	0.12	0.615	0.068	0.068	0.068
UCP2	0.03	0.04	0.04	0.03	0.03	0.00	0.574	NS	NS	NS
FAS	0.70^{AB}	0.83^{A}	0.26^{BC}	0.29^{BC}	0.05^{C}	0.07	0.017	−0.002	−0.002	−0.002
UCP3	3.39^{B}	2.46^{BC}	5.31^{A}	2.34^{BC}	0.99^{C}	0.25	0.001	−0.011	−0.010	−0.009
CEBPa	0.08^{A}	0.05^{A}	0.09^{A}	0.05^{A}	0.02^{B}	0.00	0.002	<0.0001	<0.0001	<0.0001
PPARd	2.12^{B}	2.00^{B}	3.35^{A}	1.97^{B}	0.82^{C}	0.11	<0.0001	−0.004	−0.003	−0.003
HSL	0.62	0.59	0.59	0.44	0.61	0.05	0.738	NS	NS	NS
Sirt	0.78^{A}	0.67^{A}	0.70^{A}	0.28^{B}	0.16^{B}	0.04	<0.0001	<0.0001	<0.0001	<0.0001
Muscle BF										
I	1.24^{B}	3.08^{A}	0.93^{B}	0.70^{B}	1.17^{B}	0.23	0.025	NS	NS	NS
IIa	1.23	1.47	0.77	0.68	0.86	0.10	0.092	NS	NS	NS
IIx	0.81	0.52	0.63	0.69	0.61	0.06	0.761	NS	NS	NS
IIb	5.50	1.03	0.57	1.04	0.59	0.61	0.082	NS	NS	NS
PGCa	0.86	1.91	1.32	0.34	0.62	0.14	0.058	NS	NS	NS
UCP2	1.17^{b}	2.42^{a}	1.00^{b}	0.86^{b}	0.68^{b}	0.07	<0.0001	NS	NS	NS
FAS	1.22	1.92	0.72	0.57	0.94	0.12	0.052	NS	NS	NS
HSL	1.22	0.92	0.97	0.56	0.61	0.11	0.308	NS	NS	NS
LPL	1.16^{B}	2.23^{A}	1.28^{B}	1.21^{B}	1.64^{AB}	0.10	0.011	NS	NS	NS
AMPK	1.05^{A}	0.81^{AB}	0.85^{AB}	0.60^{B}	0.59^{B}	0.05	0.033	NS	NS	NS
Sirt	1.06	0.98	1.02	0.76	1.16	0.05	0.173	NS	NS	NS
SREBP	1.12^{A}	0.64^{B}	0.18^{C}	0.37^{BC}	0.66^{B}	0.06	0.000	−0.001	−0.001	−0.001
ACC	1.02^{B}	1.55^{A}	1.06^{B}	1.12^{B}	1.12^{B}	0.05	0.015	NS	NS	NS
CEBPa	0.95	0.94	0.94	0.94	1.12	0.07	0.899	NS	NS	NS
FOX1	0.92^{AB}	1.18^{A}	0.92^{AB}	0.73^{B}	0.87^{B}	0.04	0.046	NS	NS	NS

LD = longissimus dorsi; BF = biceps femoris; *FAS* = fatty acid synthase; *ACC* = acetyl coenzyme A carboxylase; *MyHC* = myosin heavy chain; *I* = slow-oxidative type, *IIa* = fast-oxidative type, *IIx* = fast oxidative-glycolytic type, *IIb* = fast-glycolytic type of myofibers. *SREBPa* = sterol regulatory element-binding protein 1c; *FOX1* = forkhead box O1; *Sirt* = silent mating type information regulation 2 homolog; *PPARd* = peroxisome proliferative activated receptor, delta; *HSL* = hormone-sensitive triglyceride lipase; *LPL* = lipoprteinlipase; *CEBPa* = CCAAT/enhancer binding protein alpha; *AMPK* = adenosine 5′-monophosphate (AMP)-activated protein kinase; *PGCa* = peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *UCP2* = uncoupling protein 3; *UCP3* = uncoupling protein 3.
[A, B, C] Mean values within a row with different superscripts differ significantly at $P < 0.05$.
[a, b, c] Mean values within a row with different superscripts differ significantly at $P < 0.01$.
[1] DE3050, DE3100, DE3150, DE3200, DE3250 represent diet with digestive energy 3,050, 3,100, 3,150, 3,200, 3,250 kcal/kg respectively. NS means that no variable met the 0.15 significance level for entry into the model when regression analysis (REG) procedure run; + means positive effect; − means negative effect.

Fatty acid composition is associated with overall pig fatness and the fat content of muscles (Wood et al., 2008). Fatty acid composition changes with restricted feeding (Skiba et al., 2012). The SFA increased and PUFA decreased when ME decreased from 13.82 to 13.40 MJ/kg in finishing pigs (Fang et al., 2019). The present study also showed higher C16:1 and lower C14:0, C17:0 in LD muscle in higher energy diets compared with lower energy diets. Patterns of fatty acid deposition may differ across muscles (Leseigneur-Meynier and Gandemer, 1991). Pigs receiving a medium energy diet had lower proportions of C14:0, but higher C18:3n6 and C20:3n6 in BF muscle compared with those offered a high energy diet in the present study. This finding is similar to early reports which showed that compared to those fed ad libitum, 25% restricted-fed pigs had lower proportions of SFA and MUFA and higher proportions of PUFA in the fatty acid profile in muscle longissimus thoracis (Więcek et al., 2011). This is a favorable change because polyunsaturated fatty acids cannot be synthesized in the pigs' body, they must be supplied through the diet (Enser et al., 2000). A positive correlation exists between the PUFA content and the nutritional value of meat (Duan et al., 2014). Although total PUFA level was not affected by dietary energy level in the present study, a negative trend was found between PUFA proportion and energy level. Compared to ad libitum, 25% restricted-fed pigs were characterized by higher n6 PUFA and n3 PUFA proportions in muscle longissimus thoracis and muscle semimembranosus (Więcek et al., 2011). Difference in PUFA content may be due to the differences in oxidative properties observed between muscles (Andrés et al., 2001). High n3 PUFA increase the susceptibility of lipids to peroxidation in muscle (Shmookler Reis et al., 2011). Peroxidation products such as reactive oxygen species can damage muscles. Superoxide dismutase (SOD) in the mitochondria requires Mn but extracellular and cytosolic SOD in hepatic cells need Cu and Zn to function (Gropper and Smith, 2009). Both Cu and Mn retained in LD muscle decreased linearly with dietary energy level. Copper (Cu) has been mobilized to build antioxidant defenses for peroxidation products. Further, in order to get higher dietary DE, the amount of crude fiber decreased. A previous report shows that dietary fiber intake has a significant positive association with relative bone mineral content in adults aged 40 years and older (Frampton et al., 2021). Pigs that received the diet with moderate energy levels had high n6 PUFA accompanied by high Cu concentration.

5. Conclusion

In conclusion, dietary energy level may be reduced by 200 kcal/kg over a short period without affecting growth performance of Xiangcun Black pigs. Longissimus dorsi and BF muscles respond differently to dietary energy level. Meat quality of BF muscle could be improved by increasing C18:3n6 and C20:3n6 proportions and increasing fiber cross-sectional area. Likewise, more glycogen and Cu were deposited in LD muscle when pigs were fed diets with medium energy levels.

Author contributions

Yulong Yin, Ruilin Huang, Fengna Li organized the experiment and gave some advice on experiment idea. **Can Yang** conducted the animal experiment and wrote the manuscript. **Can Yang, Wenlong Wang, Xiaowu Tang, Wenxuan Su, Chaoyue Wen** conducted the experimental analysis. **Fengna Li, Yulong Yin**, and **Jian Liu** reviewed the manuscript and gave some advice on experiment idea. All authors read and approved the final manuscript.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2022.06.006.

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