Animal Nutrition 11 (2022) 171–180

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

Dietary zero-dimensional fullerene supplementation improves the meat quality, lipid metabolism, muscle fiber characteristics, and antioxidative status in finishing pigs

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ARTICLE INFO

Article history:
Received 12 January 2022
Received in revised form
27 April 2022
Accepted 20 June 2022
Available online 17 August 2022

Keywords:
Zero-dimensional fullerenes
Lipid metabolism
Meat quality
Muscle fiber
Antioxidative

ABSTRACT

With the increasing demand for high-quality pork, more nutritional substances have been studied for the regulation of meat quality. Zero-dimensional fullerenes (C60) can modulate the biological behavior of a variety of cell lines and animals. In this study, we report the biological effects of C60 on finishing pigs at different concentrations. A total of 24 barrows (Duroc × Large White × Landrace), with an average body weight of 21.01 ± 0.98 kg, were divided into 3 groups and each treated daily with C60 (100 or 200 mg per kg feed) or a control diet until the end of the experiment. Our results showed that dietary C60 supplementation improved flesh color, marbling scores, and flavor amino acid contents of longissimus dorsi (LD) of growing-finishing pigs ($P < 0.05$). C60 improved meat quality by regulating lipid metabolism and muscle fiber morphology by mediating the expression of genes, L-lactic dehydrogenase (*LDH*), myosin heavy chain (*MyHC*) *IIa*, *MyHCIIb*, peroxisome proliferator-activated receptor γ (*PPARγ*), and fatty acid transport protein 1 (*FATP1*) ($P < 0.05$). Moreover, C60 substantially promoted the mRNA expression of antioxidant enzyme genes ($P < 0.05$), which also contributed to improving meat quality. These findings have important implications for the application of C60 in the livestock industry, especially for improving the meat quality of fattening pigs.

1. Introduction

As the demand for high-quality meat continues to increase, meat producers should produce and supply healthier, safer, and higher-quality meat to ensure the sustainable development of the meat industry. However, in the past twenty years, western pig breeds were intensively selected to achieve rapid, large and efficient muscle growth, which has led to meat quality deterioration (Lefaucheur et al., 2002). Meanwhile, research on intramuscular fat (IMF) has become increasingly frequent in the porcine fat deposition field. Many studies have shown that IMF is closely related to meat quality, affecting meat properties such as water-holding capacity, tenderness, and flavor. Furthermore, selective fat deposition can improve production efficiency and play a key role in improving meat quality (Wang et al., 2009; Hua et al., 2016; Han et al., 2021). Moreover, muscle fiber type is one of the main factors determining meat quality because it is closely related to muscle fat content

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2022.06.017

(Li et al., 2010). The increase in type IIb fiber percentage and the decrease in type I and type IIa fiber percentage are related to increases in dripping loss and brightness, which will reduce pork quality (Li et al., 2010; Guo et al., 2011; Liu et al., 2016). Several approaches have been suggested for improving meat quality: resveratrol improves meat quality by regulating the muscle fiber characteristics; eucommia ulmoides Oliver leaf polyphenol can change myofiber type and improve meat quality (Zhang et al., 2015a; Zhou et al., 2016). However, the current researches are still not enough to improve the quality of finishing pigs. Thus, alternative nutritional interventions with more beneficial effects are needed.

Zero-dimensional fullerenes (C60) is an abundant member of the fullerene family (Nakagawa et al., 2018). C60 molecules can interact with each other and with up to 34 methyl radicals. C60 can be used as an effective antioxidant and free radical scavenger for its electron donor and acceptor capability, and it does not possess any genotoxic effects on human lymphocytes or mutagenic effects in vivo and in vitro (Hao et al., 2016; Aly et al., 2018; Vereshchaka et al., 2018; Sharoyko et al., 2021). These fullerene compounds include polyhydroxy C60 (fullerol), carboxylated fullerene, and polysulfonated C60, which have been proved to block the damage from free radicals in several diseases related to oxidative stress, such as ischemia/reperfusion injury, inflammatory cell apoptosis, and neurogenic diseases (Zhou et al., 2010; Hao et al., 2016; Liao et al., 2021; Nozdrenko et al., 2021). Previous studies indicated that C60 can be effectively absorbed by the animal's gastrointestinal tract and metabolized in many organs and had no genotoxic effects (Aly et al., 2018). In addition, the C60 administered orally to rats at a very high dose had no adverse effects on growth, feed intakes, and blood and biochemical variables as well as histopathological examination. Our preliminary tests also showed that fullerenes did not adversely affect mice and could even promote weight gain of mice during the growth period, and is beneficial to the intestinal mucosal health of vomitoxin model mice (Liao et al., 2021).

Based on previous reports on C60 as a potential antioxidant therapeutic agent in cells and mice, we hypothesized that the C60 could exert a beneficial effect in oxidative damage, lipid peroxidation, and meat quality in finishing pigs. In this study, we aimed to investigate the protective roles of C60 against oxidative stress and clarify the effects of C60 on the meat quality, lipid metabolism and muscle fiber regulation.

2. Materials and methods

2.1. Animal ethics statement

The experimental procedures were approved by the Protocol Management and Review Committee of the Institute of Subtropical Agriculture, Chinese Academy of Science (No. 20200708), and conducted according to the Institute of Subtropical Agriculture guidelines on Animal Care (Changsha, China).

2.2. Dietary treatments

The 3 groups were the control group (basal diet) and the experimental groups (basal diet supplemented with C60 at 100 or 200 mg per kilogram of feed). The basal diet was powdered and meets the NRC (2012) recommendations for the nutritional needs of fattening pigs (Table 1). According to a certain proportion, the C60 livestock and poultry feed additive was accurately weighed and mixed together with the basic feed for 120–180 s, and the coefficient of variation was less than 5%. C60 was provided by Xiamen Funano New material Technology Co., Ltd (Xiamen, China).

Table 1
Ingredients and nutrient levels of experimental diets (as-fed basis, %).

Item	Content
Ingredients	
Corn	66.88
Soybean meal	23.90
Wheat bran	6.00
Soybean oil	0.88
Calcium hydrophosphate	0.50
Limestone	0.54
NaCl	0.30
Premix[1]	1.00
Total	100.00
Nutrient levels[2]	
DE, MJ/kg	14.47
ME, MJ/kg	13.44
Crude protein	16.04
SID Lys	0.73
SID (Met + Cys)	0.51
SID Thr	0.52
SID Trp	0.17
SID Ser	0.72
SID Gly	0.61
Total Ca	0.51
Total P	0.45
Available P	0.20

DE = digestible energy; ME = metabolizable energy; SID = standardized ileal digestibility.

[1] Supplied per kilogram of diet: vitamin A, 10,800 IU; vitamin D_3, 4,000 IU; vitamin E, 40 IU; vitamin K_3, 4 mg; vitamin B_1, 6 mg; vitamin B_2, 12 mg; vitamin B_6, 6 mg; vitamin B_{12}, 0.05 mg; biotin, 0.2 mg; folic acid, 2 mg; niacin, 50 mg; D-calcium pantothenate, 25 mg; Cu (as copper sulfate), 25 mg; Fe (as ferrous sulfate), 100 mg; Mn (as manganese oxide), 40 mg; Zn (as zinc oxide), 80 mg; I (as potassium iodide), 0.5 mg; and Se (as sodium selenite), 0.3 mg.

[2] Values of DE, ME, SID amino acids, and aavailable P were calculated, while the others were measured.

2.3. Animals and husbandry

Twenty-four barrows (Duroc × Large White × Landrace) at 57 d old were obtained from the breeding farm in Yiyang, China. Pigs (21.01 ± 0.98 kg, mean ± SEM) were randomly assigned to 3 groups based on a completely randomized design. Each pig has a separate identification, an ear tag. The experimental period lasted for 90 d, and all pigs were housed in groups on a concrete slat floor (3.0 m × 4.0 m) with free access to water and feed throughout the experiment. The total amount of feed fed per day is at least 5% of the previous day's body weight, and the recommended feeding amount of the present invention ensures that the piglets are completely fed.

2.4. Performance measurement and sampling

On d 30 of the trial, the average feed intake and the ratio of feed to weight gain were calculated and blood samples were collected. At the end of the experiment, BW of pigs was measured on d 147. Blood samples were taken intravenously after an overnight fast (12 h), then placed at room temperature for 2 h and centrifuged at 3,000 × *g* for 10 min to separate serum. All serum was stored at - 80 °C for further analysis. All pigs were slaughtered by electrical stunning and exsanguination in a commercial abattoir. Then, samples of the LD between the 6th and 7th ribs were collected and immediately refrigerated at 2 to 4 °C for the measurement of meat quality data. Meanwhile, about 150 g of LD samples were frozen at - 20 °C for lyophilization and muscle chemical analysis. Besides, approximately 1-cm-thick LD samples were frozen in liquid nitrogen for the genes and proteins expression analysis.

2.5. Meat quality analysis

The LD samples were used in the following order: 1) 3.0-cm-thick chop used for objective color (lightness, L*; redness, a*; and yellowness, b*), and pH measurement; 2) 5.0-cm-thick chop used for water-holding capacity (WHC) measurement. The pH values at 45 min, 12, 24, and 48 h post-mortem were determined by a hand-held pH meter (pH-STAR, SFK-Technology, Denmark). After cutting for 10 min, the flesh color of LD was measured by a hand-held colorimeter (CR-410, Minolta Camera, Co., Osaka, Japan). The WHC was determined according to the method in the references (Li et al., 2018). The meat size was partially modified to reduce the test error. The LD (2 cm × 3 cm × 5 cm, about 35 g) on the left side of the carcass after slaughter for 24 h (4 °C) was taken and weighed (MW1; MW1 stands for the meat weight at first time weighing). The LD muscle sample was hooked with an S-hook, then loaded into a plastic bag and inflated sealed. After being suspended at 4 °C condition for 48 h, samples were weighed again (MW2). The drip loss was calculated according to the following formula: Drip loss (%) = (MW1 - MW2)/MW1 × 100.

2.6. Serum biochemical indexes measurements

The serum biochemical indexes, creatine kinase (CK), total cholesterol (CHO), low-density lipoprotein cholesterol (LDL), and high-density lipoprotein cholesterol (HDL), were measured with an automatic biochemical analyzer Chemray 240/800 (Ray to Life Sciences Co., Ltd, Shenzhen, China) and the commercial kits were purchased from Changchun Huili Biotech Limited (Changchun, China).

2.7. Free amino acids measurements

The methods for determining amino acids in serum and LD muscle were summarized below. Firstly, the samples were melted at 4 °C, and the vortex lasted 10 s. Secondly, the sample (10 μL) was taken into a 1.5-mL centrifuge tube, and 10 μL water, 5 μL internal standard, 40 μL isopropyl alcohol (1% formic acid) were added to it and mixed for 60 s, and then the samples were centrifuged at 13,000 × *g* at 4 °C for 5 min. Subsequently, 10 μL of the supernatant solution was placed in a 1.5-mL centrifuge tube, and the derivative reagent was added at 50 °C for 10 min. Approximately 50 mg of freeze-dried LD was homogenized in 10 mL of 0.01 mol/L methyl alcohol and shaken for 60 min. After centrifuging at 13,000 × *g* at 4 °C for 5 min, 10 μL of the supernatant was mixed with 10 μL water, 5 μL internal standard, and 40 μL isopropyl alcohol into a 1.5-mL centrifuge tube and shaken for 60 min, centrifuged again. The supernatant (10 μL) was chosen to mix with 70 μL PBS, and the derivative reagent was added at 50 °C for 10 min. All samples were diluted 10 times with RNase-free water after derivation and waiting for a test. All samples were analyzed using a liquid chromatography analyzer (UPLC I-Class, Waters, USA) and mass spectrometer (XEVO TQ-XS, Waters, USA).

2.8. Fatty acid composition and intramuscular fat measurements

The fatty acid composition of LD muscle was measured as previously (Zhong et al., 2021). Approximately 50 mg of freeze-dried muscle tissue was homogenized, and 3 mL n-hexane was added and shaken at 50 °C for 30 min. And then, 3 mL KOH methanol solution was added (0.4 mol/L) and shaken at 50 °C for 30 min. Subsequently, 1 mL of water was added and mixed well, the upper layer was taken by standing stratification, and the sample was tested by temperament injection with a mass spectrometer (GC–MS 7890B-5977A, Agilent Technologies Inc. California, USA). The tank temperature was initially at 50 °C for 1 min, increased by 25 °C/min to 175 °C, and 4 °C/min to 230 °C, and it was 24.75 min in total.

Intramuscular fat was determined using a fully automated fat analyzer SOX416 (Gerhardt Co. Ltd, German). The brief steps were as follows: first, a Soxhlet extraction bottle was dried at 105 ± 2 °C to constant weight; second, a lyophilized muscle sample was taken and weighed (MW1), and wrapped in filter paper and weigh it again (MW2); third, the filter paper packages was put into the suction tube, and anhydrous ether was added to the extraction bottle, and it was heated in a water bath at 60–75 °C, and it was repeated 70 times until the end of the extraction; final, the samples package was put out and dried at 105 ± 2 °C to constant weight (MW3). The content is calculated according to the following formula: IMF (%) = [(WM2 − WM3) × DM]/(WM2 − MW1) × 100, where DM refers to the dry matter content of sample.

2.9. Antioxidant enzyme and malondialdehyde (MDA) content measurements

The levels of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and total antioxidative capacity (T-AOC) in serum were determined with commercial ELISA kits (Meimian, Jiangsu, China) according to manufacturer instructions. The MDA contents were assayed using spectrophotometric methods (Liao et al., 2020).

2.10. Relative genes expression analyzed by quantitative real-time polymerase chain reaction (RT-PCR)

RT-PCR was followed by the previous one (Tang et al., 2022). In brief, extracted total RNA using the Trizol reagent (Thermo Fisher Scientific, USA), purified extracted RNA and constructed cDNA Synthesis using the Evo M-MLV RT Kit with gDNA Clean for qPCR (Accurate Biotechnology Co., Ltd.). The quality and quantity of the total RNA were determined with a Nanodrop 2000 Spectrophotometer (Thermo Scientific, Courtaboeuf, France). Amplification conditions were performed as previously (Tang et al., 2022), and the primers used in this experiment are as shown in Table 2. Primers specific to PCR templates were designed with Primer 3, while primers for *GPX*, *ACC*, *FAS*, *FAT/CD36*, *PPARγ*, *FATP1*, *SREBP1c*, and *CPT1B-1* genes were as previously described (Zhong et al., 2021).

2.11. Cross-sectional area (CSA) and fiber density determination

The CSA of myofibers in LD was measured as previously (Zhang et al., 2015a). In brief, LD samples were cut perpendicular to the direction of the muscle fibers, and 10 μm serial tissue sections were excised using Leica CM1850 cryostat (Leica Instrument GmbH, Germany). The sections were air-dried at 25 °C for 18 min, and then H&E staining was performed. Five fascicles were randomly selected from each section, and the images of 5 consecutive sections were analyzed by light microscope (Olympus, Tokyo, Japan) and camera (JVC, Yokohama, Japan) at 40 times magnification, and the myofiber density of LD was measured using Image-Pro Plus 4.5 (Silver Spring, MD, USA) and the CSA calculated.

2.12. Data analysis

All the data were analyzed by a one-way ANOVA model, followed by Duncan's multiple range tests by windows statistical software package SPSS 20.0 (SPSS Inc., Chicago, IL, USA), and significant differences between means were determined. Results on

Table 2
Primers are used for quantitative real-time PCR.

Gene	Primers	Sequence (5′ to 3′)
MyHCI	Forward	GAGCGAAAGCATTTGCCAAG
	Reverse	GGCATCGTTTATGGTCGGAAC
MyHCIIa	Forward	AAGGGCTTGAACGAGGAGTAGA
	Reverse	TTATTCTGCTTCCTCCAAAGGG
MyHCIIx	Forward	GCTGAGCGAGCTGAAATCC
	Reverse	ACTGAGACACCAGAGCTTCT
MyHCIIb	Forward	AGAAGATCAACTGAGTGAACT
	Reverse	AGAGCTGAGAAACTAACGTG
LDH	Forward	ATGAAGAGGAACCACATTA
	Reverse	TTATTGCCTCAGTAGCTTG
GPX	Forward	ACAGTGCTGACACTCTGTGG
	Reverse	CTGGGAGCCCACATTCACAT
ACC	Forward	TTCCAGGCACAGTCCTTAGG
	Reverse	TCATCCAACACGAGCTCAGT
FAS	Forward	CTACCTTGTGGATCACTGCATAGA
	Reverse	GGCGTCTCCTCCAAGTTCTG
HSL	Forward	GCAGCATCTTCTTCCGCACA
	Reverse	AGCCCTTGCGTAGAGTGACA
FAT/CD36	Forward	CTGGTGCTGTCATTGGAGCAGT
	Reverse	CTGTCTGTAAACTTCCGTGCCTGTT
PPARγ	Forward	AGGGCCAAGGATTCATGACA
	Reverse	GTGGTTCAACTTGAGCTGCA
FATP1	Forward	GGAGTAGAGGGCAAAGCAGG
	Reverse	AGGTCTGGCGTGGGTCAAAG
SREBP1c	Forward	GCGACGGTGCCTCTGGTAGT
	Reverse	CGCAAGACGGCGGATTTA
GAPDH	Forward	AAGGAGTAAGAGCCCCTGGA
	Reverse	TCTGGGATGGAAACTGGAA
CPT1B-1	Forward	ATGGTGGGCGACTAACT
	Reverse	TGCCTGCTGTCTGTGAG
Actin	Forward	ATGGTGAAGGTCGGAGTGAAC
	Reverse	CTCGCTCCTGGAAGATGGT
GPX1	Forward	ATTCACTCGGCATCCATTGC
	Reverse	GCTTGGCAAAATGGAATCTAGT
GPX4	Forward	GCTCCATGCACGAATTCTCAG
	Reverse	AAGGCCAGAATCCGTAAACCA
CAT	Forward	GAAGCATTGGAAGGAGCAGC
	Reverse	TGGCATGCACAACTCTCTCA
CuZnSOD	Forward	TCGAGCTGAAGGGAGAGAAGA
	Reverse	CACATTGCCCAGGTCTCCAA
MnSOD	Forward	TGCCGTACGACTATGGCG
	Reverse	TTGATGTGGCCTCCACCGTT

MyHC = myosin heavy chain; *LDH* = lactate dehydrogenase; *GPX* = glutathione peroxidase; *ACC* = acetyl-CoA carboxylase; *FAS* = fatty acid synthase; *HSL* = hormone-sensitive lipase; *FAT/CD36* = fatty acid translocase; *PPARγ* = peroxisome proliferator-activated receptor γ*;* *FATP1* = fatty acid transport protein 1; *SREBP1c* = sterol regulatory element binding protein-1c; *GAPDH* = glyceraldehyde-3-phosphate dehydrogenase; *CPT1B-1* = carnitine palmitoyl transferase 1B; *CAT* = catalase; *CuZnSOD* = copper-zinc-superoxide dismutase; *MnSOD* = manganese superoxide dismutase.

the column chart were expressed as the mean ± standard error, and other results in the tables are presented as mean and SEM. Differences were declared significant at $P < 0.05$, and trends toward significance at $0.05 \le P < 0.10$.

3. Results

3.1. Performance

The final weight and average daily gain of the pigs fed with C60 were not significantly different from those of the control group (Table 3). In addition, no significant difference was observed in the average daily feed intake. However, feeding with C60 (0.2%) significantly increased the ratio of feed intake to body weight gain compared to the control ($P < 0.01$). The addition of C60 improved the growth performance to a certain extent.

Table 3
Effect of dietary zero-dimensional fullerenes (C60) supplementation on growth performance of the finishing pigs ($n = 6$).[1]

Item, kg	CON	0.1% C60	0.2% C60	SEM	*P*-value
IBW	20.38	21.38	21.40	0.98	0.89
BW, d 30 of the trial	42.23	44.48	47.92	1.76	0.44
ADG, d 30 of the trial	0.73	0.77	0.88	0.04	0.28
ADFI, d 30 of the trial	1.11	1.12	1.12	0.20	0.86
F/G, d 30 of the trial	2.27^{a}	2.19^{b}	1.99^{c}	0.12	<0.01
FBW	102.67^{c}	112.60^{b}	121.17^{a}	9.43	<0.01

IBW = initial body weight; BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; F/G = the ratio of ADFI to ADG; FBW = final body weight.

[a,b,c] Values in a row with superscripts differ significantly ($P < 0.05$).

[1] Results in tables are presented as mean and SEM.

3.2. Serum biochemical indexes

The concentrations of serum metabolites in the different dietary treatments are shown in Table 4. The diet supplemented with 0.1% or 0.2% C60 did not affect the concentrations of CHO, HDL, LDL, and CK in the serum of the pigs.

3.3. Meat quality

The meat quality index is shown in Fig. 1. Compared to the control group, dietary 0.2% C60 supplementation increased the pH_{3h} (Fig. 1A) and flesh color (Fig. 1B), and marbling scores tended to increase in the C60 group ($P > 0.05$). Meat color scores in the control group were around score 4, while 33% of pigs in the 0.1% C60 group scored 5, and 50% of pigs in the 0.2% C60 group scored greater than or equal to 5. In the control group, 33% of the pigs had marbled scores less than or equal to score 2, while all the pigs with 0.2% C60 had marbled scores greater than or equal to 3, and 66% of the pigs had marbled scores of 4. It can also be seen from Fig. 1D that the pigs supplemented with C60 had more marbling than the control group. The mRNA expression level of the L-lactic dehydrogenase (*LDH*) gene is shown in Fig. 1C. C60 downregulated the mRNA expression level of *LDH* ($P < 0.05$).

Further analysis was performed with a flesh color meter (Table 5). In pigs with 0.2% C60, yellowness increased ($P < 0.05$), but there was no difference with the control after 12 h. After slaughter, the drip loss of pigs fed 0.1% and 0.2% fullerene respectively decreased by 8.60% and 13.98% compared with the pigs in the control group ($P < 0.05$). There has been an increase in IMF content.

3.4. Fatty acid profile in skeletal muscle

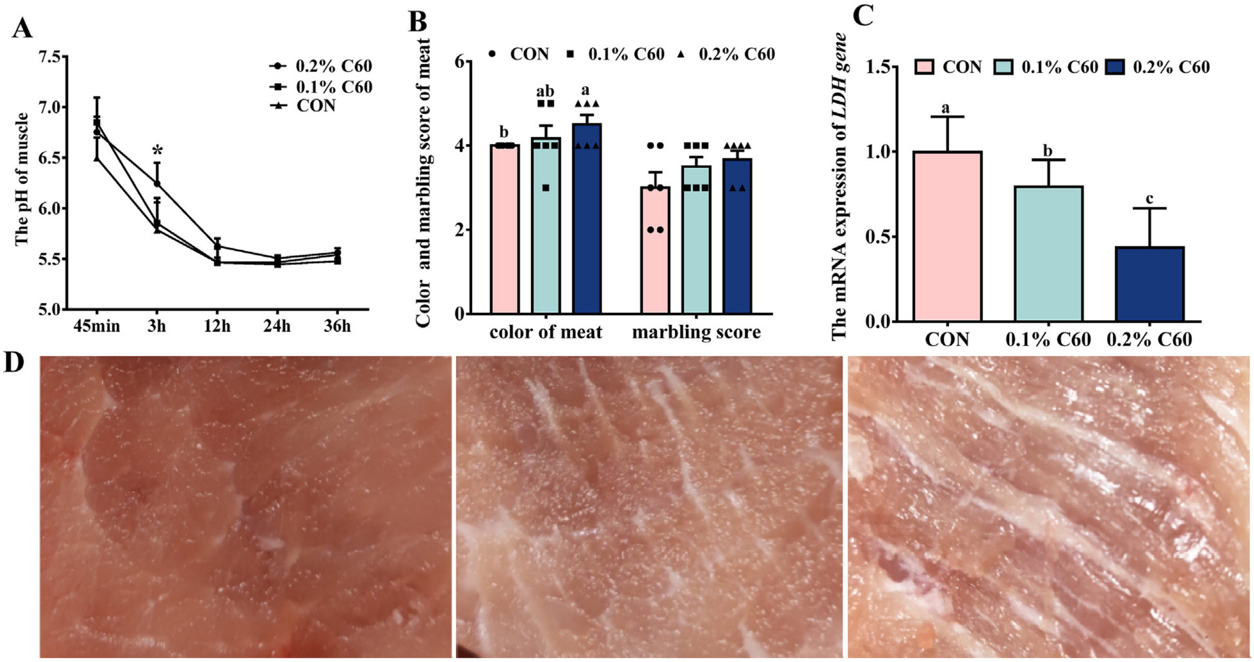
The fatty acid composition of the LD muscle is presented in Table 6. Dietary 0.2% C60 decreased the concentration of saturated fatty acids (SFA) as C15:0, C15:0, C17:0, C20:4n6, C23:0 ($P < 0.05$). While the SFA of pigs fed 0.1% and 0.2% fullerene respectively

Table 4
Impact of diet supplemented with zero-dimensional fullerenes (C60) on serum biochemistry of finishing pigs ($n = 6$).[1]

Item	CON	0.1% C60	0.2% C60	SEM	*P*-value
CHO, mmol/L	2.49	2.44	2.46	0.08	0.96
HDL, mmol/L	1.01	0.94	0.95	0.04	0.59
LDL, mmol/L	0.21	0.32	0.34	0.03	0.10
CK, U/L	4,466.25	3,564.28	3,789.48	418.10	0.69

CHO = total cholesterol; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; CK = creatine kinase.

[1] Results in tables are presented as mean and SEM.



CON 0.1% C60 0.2% C60

Fig. 1. Effect of dietary zero-dimensional fullerenes (C60) supplementation on meat quality of finishing pigs ($n = 6$). (A) The pH values of longissimus dorsi muscle of finishing pigs at 45 min, 3, 12, 24, and 36 h post−slaughter; *$P < 0.05$ vs. control. (B) The color and marbling score of meat. (C) The mRNA expression of L-lactic dehydrogenase (*LDH*) gene. (D) Representative image of the longissimus dorsi muscle. Results on the column chart were expressed as the mean ± standard error. [a, b, c] Bars with different letters were declared significant at $P < 0.05$.

Table 5
Impact of diet supplemented with zero-dimensional fullerenes (C60) on meat quality of finishing pigs ($n = 6$).[1]

Item	Time	CON	0.1% C60	0.2% C60	SEM	*P*-value
L*	3 h	48.1	48.68	49.15	0.95	0.91
	12 h	47.62	49.08	49.02	0.65	0.70
	24 h	51.31	50.62	52.65	0.76	0.58
	36 h	49.42	48.11	50.52	1.44	0.83
a*	3 h	17.65	17.51	18.64	0.74	0.81
	12 h	19.55	18.68	19.77	0.66	0.81
	24 h	20.07	19.26	20.55	0.37	0.37
	36 h	49.42	48.12	50.52	0.82	0.45
b*	3 h	4.41^b	4.86^b	6.31^a	0.30	0.02
	12 h	7.35	8.05	8.87	0.39	0.36
	24 h	9.52	9.24	10.41	0.36	0.41
	36 h	10.93	10.37	12.15	0.49	0.31
Drip loss, %		3.65^a	2.25^b	2.05^b	0.005	0.02
Intramuscular fat, %		1.75	2.05	2.25	0.003	0.05

L* = lightness; a* = redness; b* = yellowness.
[a,b] Values in a row with superscripts differ significantly ($P < 0.05$).
[1] Results in tables are presented as mean and SEM.

decreased by 7.02% and 10.87% compared with the pigs in the control group. C60 can affect the free fatty acid (FAA) components, which play a role in meat flavor generation and value of nutritional.

3.5. Free amino acid profiles in serum and skeletal muscle

As shown in Table 7, the concentration of amino acids in the serum did not change significantly. The addition of 0.2% C60 increased serum threonine and lysine by 13.43% and 11.10%, respectively, compared to the control, but the difference was not significant. In addition, the serum glycine concentration of the pigs fed with C60 was tended lower than that of the control group.

The free amino acid profiles of the LD muscle are presented in Table 8. Compared to the control group, the addition of 0.2% C60 increased the content of most amino acids ($P < 0.01$), especially the flavor amino acids such as glutamate, tyrosine, phenylalanine, alanine, and glycine, which increased by 34.86%, 34.02%, 30.11%, 18.24%, and 17.88%, respectively. Moreover, threonine, methionine, and leucine increased by 21.20%, 28.73%, and 41.61%, respectively. Serine concentration showed an increase, albeit insignificant, a trend in the 0.2% C60 group compared to the control group ($P > 0.05$). In addition, the total amino acid (TAA) in the 0.2% C60 group was higher than that in the other 2 groups ($P < 0.01$). Adding C60 to feed improves the flavor of meat by increasing the flavor amino acids.

3.6. Effect of dietary C60 supplementation on fiber type and fatty acid metabolism of finishing pigs

Compared to the control, dietary C60 supplementation resulted in greater myosin heavy chain (*MyHC*) *IIa* mRNA levels and lower *MyHCIIb* mRNA levels ($P < 0.05$, Fig. 2A). The genes expression related to muscle lipid metabolism was analyzed (Fig. 2B and C), including fatty acid translocase (*FAT/CD36*), acetyl-CoA carboxylase (*ACC*), fatty acid transport protein 1 (*FATP1*), fatty acid synthase (*FAS*), peroxisome proliferator-activated receptor γ (*PPAR*γ), and the transcription factor sterol regulatory element-binding protein-1c (*SREBP1c*). Compared to the control group, 0.2% C60 increased the mRNA expression levels of *PPAR*γ and *FATP1* ($P < 0.05$), whereas there were no significant differences observed in the levels of *FAT/CD36, CPT1B, ACC, SREBP1c*, and *FAS* in the present study (Fig. 3B and C). Additionally, C60 downregulated the mRNA expression level of *HSL* ($P < 0.05$). Above, C60 alters the myofiber type and fatty acid regulatory gene expression.

Table 6
Impact of dietary zero-dimensional fullerenes (C60) on longissimus dorsi fatty acid content of finishing pigs ($n = 6$).[1]

Fatty acids, μg/g	CON	0.1% C60	0.2% C60	SEM	P-value
C6:0	2.90	2.87	2.80	0.07	0.85
C8:0	4.70	4.99	5.25	0.47	0.90
C10:0	53.03	52.58	59.64	5.63	0.87
C12:0	39.00	39.82	43.81	4.55	0.91
C14:0	737.73	754.99	842.35	88.89	0.89
C15:0	15.96	21.22	12.62	1.59	0.07
C15:1	1.98^{a}	2.02^{a}	1.79^{b}	0.04	<0.01
C16:0	14,018.79	14,761.79	15,743.47	1,534.70	0.91
C16:1	1,810.64	1,703.60	2,195.27	178.60	0.53
C17:0	90.97^{ab}	128.44^{a}	75.07^{b}	10.59	0.10
C17:1	73.50	101.34	69.73	8.76	0.29
C18:0	7,326.72	8,078.54	7,930.72	802.07	0.93
C18:1n9c	21,816.98	22,598.51	25,489.98	2,449.46	0.83
C18:2n6c	5,578.75	5,539.69	4,693.46	367.74	0.57
C18:3n6	38.22	37.87	35.59	0.80	0.37
C18:3n3	117.37	123.45	107.73	11.01	0.86
C20:0	83.34	96.84	98.64	11.08	0.85
C20:1	437.38	343.50	427.57	68.68	0.85
C20:2	211.17	215.18	188.74	18.57	0.84
C21:0	8.83	8.70	7.56	0.37	0.34
C20:3n6	131.48	128.06	110.82	6.50	0.41
C20:4n6	1,002.22^{a}	935.44^{ab}	802.04^{b}	37.99	0.08
C20:3n3	50.53	49.15	46.45	2.35	0.80
C22:0	15.96	16.60	15.65	0.33	0.52
C23:0	8.94^{a}	8.64^{b}	8.08^{c}	0.14	0.02
C24:0	5.82	4.79	4.15	0.47	0.36
C22:6	11.18	10.46	8.70	0.64	0.28
C24:1	21.86	21.64	18.12	1.31	0.46
SFA[2]	22,313.07	23,880.54	24,738.33	2,438.42	0.93
MUFA[3]	24,162.34	24,770.62	28,202.40	2,686.42	0.83
PUFA[4]	6,929.76	6,824.12	5,804.79	417.71	0.51
PUFA:SFA	0.34	0.31	0.25	0.08	0.22

SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid.

[a,b,c] values in a row with superscripts differ significantly ($P < 0.05$).

[1] Results in tables are presented as mean and SEM.

[2] SFA = C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C23:0 + C24:0.

[3] MUFA = C14:1 + C16:1 + C18:1n9t + C18:1n9c + C20:1+ C24:1.

[4] PUFA = C18:1n9c + C18:2n6c + C18:3n6 + C18:3n3 + C20:2 + C20:3n6 + C20:4n6 + C20:3n3 + C22:6n3.

Table 7
Impact of dietary zero-dimensional fullerenes (C60) on serum free amino acids of finishing pigs ($n = 6$).[1]

Amino acid, mg/mL	CON	0.1% C60	0.2% C60	SEM	P-value
EAA					
Histidine	53.95	54.78	54.91	1.36	0.96
Threonine	124.72	141.48	128.46	3.96	0.20
Lysine	109.57	121.73	124.22	7.28	0.71
Methionine	30.24	31.76	30.32	0.82	0.72
Valine	202.16	218.34	218.75	7.38	0.61
Leucine	131.68	148.03	146.02	5.16	0.39
Phenylalanine	74.34	77.56	77.94	1.65	0.65
Isoleucine	80.24	94.26	95.27	3.95	0.23
NEAA					
Arginine	75.92	80.99	84.70	3.78	0.66
Asparaginase	37.73	38.37	37.15	0.77	0.83
Glutamine	234.98	237.44	228.35	8.60	0.92
Serine	72.97	70.94	73.17	2.13	0.90
Glycine	439.07	404.43	418.69	15.72	0.69
Aspartic acid	6.75	7.27	7.77	0.70	0.85
Glutamic acid	99.64	107.35	101.18	4.44	0.78
Alanine	368.37	358.61	350.02	13.35	0.87
Proline	167.48	151.73	144.50	7.63	0.48
Cysteine	20.18	21.44	20.19	0.80	0.78
Tyrosine	62.12	70.14	68.05	2.51	0.42
Tryptophan	70.23	72.91	74.63	1.85	0.65

EAA = essential amino acids; NEAA = non-essential amino acids.

[1] Results in tables are presented as mean and SEM.

Table 8
Impact of dietary zero-dimensional fullerenes (C60) on longissimus dorsi free amino acids of finishing pigs ($n = 6$).[1]

Amino acid, mg/g muscle	CON	0.1% C60	0.2% C60	SEM	P-value
EAA					
Histidine	53.95	54.78	54.91	1.36	0.96
Threonine	124.72	141.48	128.46	3.96	0.20
Lysine	109.57	121.73	124.22	7.28	0.71
Methionine	30.24	31.76	30.32	0.82	0.72
Valine	202.16	218.34	218.75	7.38	0.61
Leucine	131.68	148.03	146.02	5.16	0.39
Phenylalanine	74.34	77.56	77.94	1.65	0.65
Isoleucine	80.24	94.26	95.27	3.95	0.23
NEAA					
Arginine	75.92	80.99	84.70	3.78	0.66
Asparaginase	37.73	38.37	37.15	0.77	0.83
Glutamine	234.98	237.44	228.35	8.60	0.92
Serine	72.97	70.94	73.17	2.13	0.90
Glycine	439.07	404.43	418.69	15.72	0.69
Aspartic acid	6.75	7.27	7.77	0.70	0.85
Glutamic acid	99.64	107.35	101.18	4.44	0.78
Alanine	368.37	358.61	350.02	13.35	0.87
Proline	167.48	151.73	144.50	7.63	0.48
Cysteine	20.18	21.44	20.19	0.80	0.78
Tyrosine	62.12	70.14	68.05	2.51	0.42
Tryptophan	70.23	72.91	74.63	1.85	0.65

EAA = essential amino acids; NEAA = non-essential amino acids.

[1] Results in tables are presented as mean and SEM.

3.7. Effect of dietary C60 supplementation on LD fiber morphology and type composition of finishing pigs

Compared to the control group, decreased CSA was observed with dietary 0.2% C60 supplementation, as well as fiber diameter ($P < 0.05$). There was no difference in CSA between the 0.1% and 0.2% C60 groups (Fig. 3).

3.8. Serum antioxidative enzyme activities and MDA content of pigs

Compared to the control group, the 0.2% C60 group had higher SOD, GSH-Px, and T-AOC activities and had lower MDA content ($P < 0.05$) in the serum of 57d pigs (Fig. 4A). In addition, diets supplemented with 0.1% and 0.2% C60 increased the antioxidant enzyme activity of finishing pigs and decreased the MDA content (Fig. 4B). Thus, C60 can improve antioxidant status and is conducive to alleviating lipid peroxidation in finishing pigs after slaughter.

3.9. Muscle antioxidative enzyme activities, MDA content, and mRNA expression of relative genes

Dietary C60 supplementation did not affect muscle T-AOC activity (Fig. 5A) but resulted in better SOD and CAT enzyme activities ($P < 0.05$), and decreased MDA content. Moreover, dietary C60 supplementation significantly improved ($P < 0.05$) SOD, CAT, T-AOC activities, and decreased MDA content (Fig. 5B). Compared to the control group, 0.2% C60 lowered ($P < 0.05$) the relative mRNA expression levels of *GPX1*, *GPX 4*, *CAT*, CuZn*SOD*, and Mn*SOD* in LD of finishing pigs (Fig. 6).

4. Discussion

Our preliminary studies showed that C60 could promote the growth of mice during the growth period and was beneficial to the intestinal mucosal health of vomitoxin model mice (Liao et al., 2021). To further extend the scope of this application, our present study demonstrates the beneficial effects of C60 on growth and feed efficiency in pigs owing to their ability to attenuate oxidative

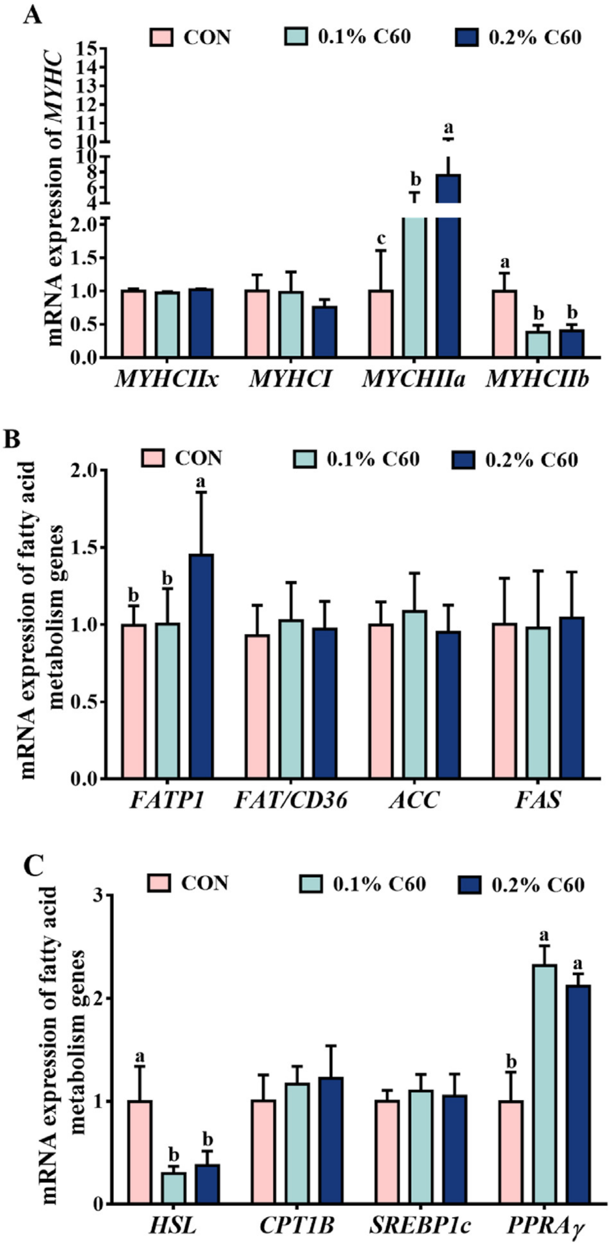


Fig. 2. Effect of dietary zero-dimensional fullerenes (C60) supplementation on fiber type and fatty acid metabolism of finishing pigs ($n = 6$). (A) The expression of the key genes, including myosin heavy chain (*MyHC*) *IIx*, *MyHC I*, *MyHC IIa*, *MyHC IIb*. (B, C) The expression of genes related to lipid metabolism. *FATP1* = fatty acid transport protein 1; *FAT/CD36* = fatty acid translocase; *ACC* = acetyl-CoA carboxylase; *FAS* = fatty acid synthase, *HSL* = hormone-sensitive lipase; *CPT1B* = carnitine palmitoyl transferase 1*B*; *SREBP1c* = sterol regulatory element-binding protein-1c; *PPARγ* = peroxisome proliferator-activated receptor γ. Results on the column chart were expressed as the mean ± standard error. $^{a, b, c}$ Bars with different letters were declared significant at $P < 0.05$.

stress. Another important finding of our experiment showed that dietary C60 supplementation improved flesh color and marbling scores and protein, fatty acid, and the flavor amino acid contents of LD in growing-finishing pigs.

We first analyzed the pH, and WHC of LD, which are the mark indexes of meat quality (Xu et al., 2020). The rate and extent of pH decline are key factors influencing the loss of water in meat, and proteolytic degradation may result in the shrinking of muscle cells and drip loss. High *LDH* activity and mRNA level are accompanied by the acceleration of glycolysis and the accumulation of lactic acid which causes the decrease of muscle pH and deterioration of meat quality (Guo et al., 2011; Díazgarcía et al., 2017). In this study, we found that dietary C60 supplementation decreased the mRNA expression of *LDH*, leading to reduce the occurrence of glycolysis in the muscle cell, thus causing a boost in the pH value of meat in growing-finishing pigs, which sustained water-holding retention, tenderness, and color of pork meat.

The fiber type composition and CSA are represented muscle fiber characteristics and related to meat tenderness. The fiber type composition is composed of 2 main fiber types: type I (slow twitch) and type II (fast-twitch) muscle fibers, and type II fibers can be further categorized into subtypes of fast-twitch oxidative (IIa), fast twitch-glycolytic fibers (IIb), and intermediate fibers (IIx), which are determined by the MyHC family (Rehfeldt et al., 2008; Guo et al., 2011; Joo et al., 2013). Previous studies indicated that a high proportion and larger area of glycolytic type IIb fibres in muscle is associated with lighter meat, larger CSA, and lower WHC in pigs (Kim et al., 2013). Therefore, it is important for us to pursue a useful approach to regulate the muscle fibers type, improving the muscle fiber characteristics and meat quality. Our results revealed that dietary C60 supplementation increased the expression level of *MyHCIIa* and reduced the level of *My*HCIIb, leading to a shift of muscle fibers from type IIB to type IIA, causing more tenderness of the meat, thus improving meat quality. More specifically, the group supplemented with 0.2% C60 exhibited a higher proportion of type IIA and IIB and lower CSA.

IMF is correlated with juiciness and flavor (Li et al., 2018). Our present study showed that C60 could increase IMF and SFA content. This result might be associated with the metabolism of fatty acids in skeletal muscle, including fatty acid biosynthesis, fatty acid oxidation, and lipid transport, which regulates the fat content of skeletal muscle (Li et al., 2018; Xu et al., 2021). Through extensive analysis of lipid metabolism-related genes, *PPARγ*, *ACC*, *FASP1c*, *FAS*, *FATP1*, *FAT*, *HSL*, and *CPT1B* (Allard et al., 2021; Stachowiak et al., 2014; Dias et al., 2015; Zhang et al., 2015b), our data demonstrated that C60 significantly upregulated the mRNA expression level of *FATP1*, indicating that C60 might increase the uptake of fatty acid. C60 also inhibited lipid catabolism *HSL* expression by *PPARγ* signaling molecules, suggesting that C60 improves fatty acid deposition in the skeletal muscle, improving pork flavor.

Lipid peroxidation is another important cause of meat spoilage, leading to the formation of various aldehyde compounds that can react with DNA, proteins, enzymes, and lipoproteins (Rajamani et al., 2021), thus affecting the function of living tissues and the quality of meat products (Baghban Kanani et al., 2017; Humam et al., 2020). Reducing lipid peroxidation and improving antioxidant status can increase the quality of meat products and shelf life. Our previous data revealed that C60 supplementation in mice diet decreased MDA concentration in serum and ROS content in liver tissues while increasing liver tissues' GSH-Px content, as well as serum SOD level (Liao et al., 2021). Similarly, this study proved that C60 acted as a potent antioxidant and free radical scavenger and significantly reduced the concentration of MDA, alleviated oxidative damage to biological tissues and meat, and enhanced the content of T-AOC, GSH-Px, as well as *GSH-Px* mRNA in the muscle, which contributed to the color and freshness of meat. Collectively, we suggested that the improved pork quality in dietary C60 treatment of this study was possibly ascribed to improved oxidative stability.

In summary, our results further expanded the application value of C60 and demonstrated that C60 not only relieved oxidative stress response and improved growth efficiency in the weaning stage but also improved meat quality, including the flesh color, IMF, flavor, and water holding capacity by mediating the LDH or MyHCIIa

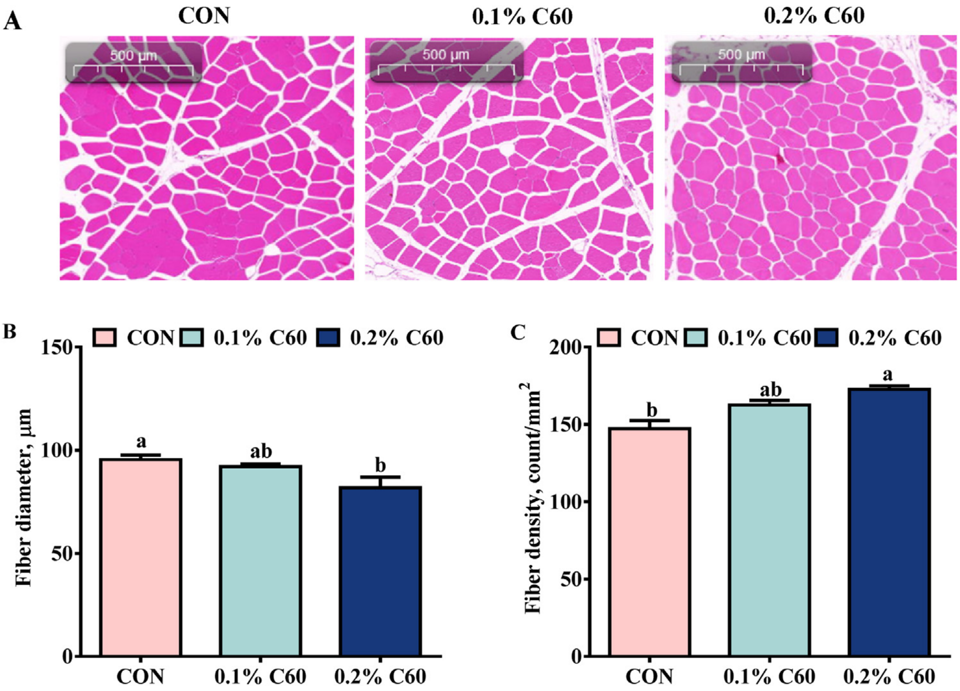


Fig. 3. Effect of dietary zero-dimensional fullerenes (C60) supplementation on longissimus dorsi (LD) fiber morphology and type composition of finishing pigs ($n = 6$). (A) Fiber morphology of LD muscle. (B) Fiber diameter of LD muscle. (C) Fiber density of LD muscle. Results on the column chart were expressed as the mean ± standard error. $^{a, b}$ Bars with different letters were declared significant at $P < 0.05$.

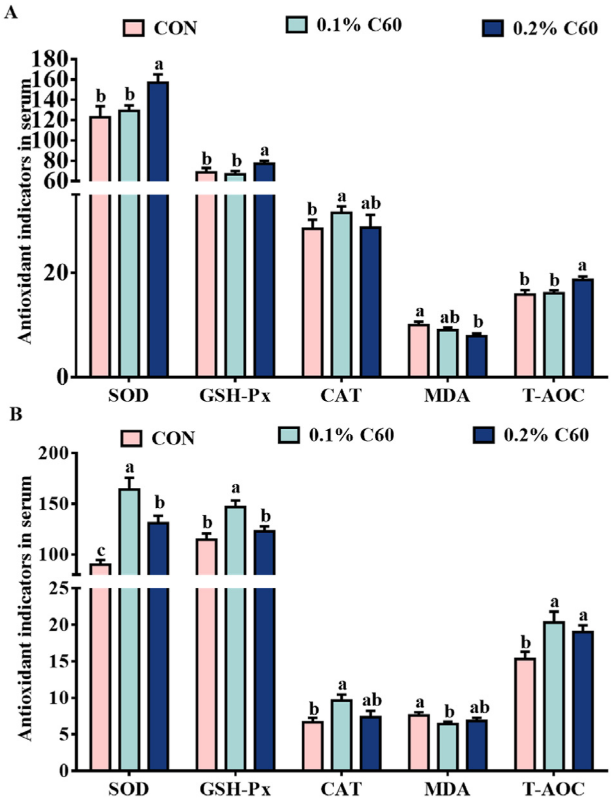


Fig. 4. Effect of dietary zero-dimensional fullerenes (C60) supplementation on antioxidative enzyme activities and malonaldehyde (MDA) content in the serum of finishing pigs ($n = 6$). (A) The antioxidative enzyme levels in serum of finishing pigs on d 30 of the trial, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px). (B) The antioxidative enzyme levels in serum of finishing pigs at the end of the trial. Results on the column chart were expressed as the mean ± standard error. $^{a, b}$ Bars with different letters were declared significant at $P < 0.05$.

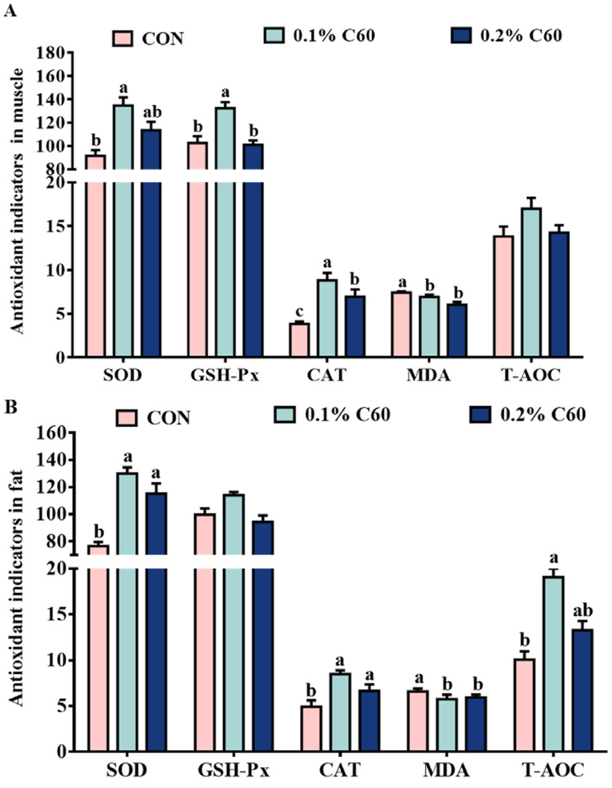


Fig. 5. Effect of dietary C60 supplementation on antioxidative enzyme activities and malonaldehyde (MDA) content in muscle and fat of finishing pigs ($n = 6$). (A) The antioxidative enzyme levels of longissimus dorsi muscle including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px). (B) The antioxidative enzyme levels of fat ($n = 6$). Results on the column chart were expressed as the mean ± standard error. $^{a, b, c}$ Bars with different letters were declared significant at $P < 0.05$.

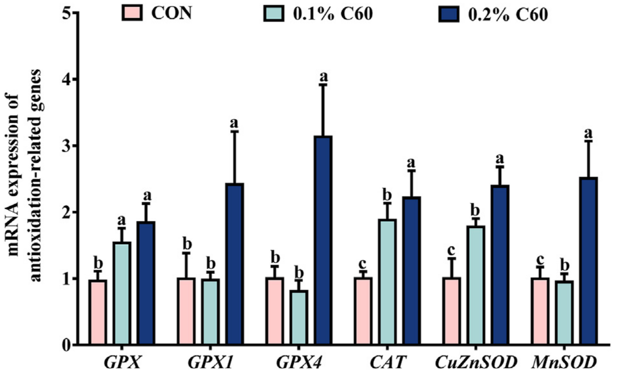


Fig. 6. Effect of adding C60 to diet on genes of glutathione peroxidase (*GPX*), *GPX1*, *GPX 4*, catalase (*CAT*), copper-zinc-superoxide dismutase (*CuZnSOD*), and manganese superoxide dismutase (*MnSOD*) mRNA expression in longissimus dorsi of finishing pigs ($n = 6$). Results on the column chart were expressed as the mean ± standard error. [a, b, c] Bars with different letters were declared significant at $P < 0.05$.

signaling pathway during the growth and fattening stages. These results will provide a significant application foundation supplemented with 200 mg C60 per kg of feed for consumers' demand for high-quality meat.

Author contributions

Simeng Liao: Data curation, Writing-Original draft preparation. **Guang Liu:** Visualization, Investigation. **Bie Tan:** Supervision. **Ming Qi:** Software, Visualization. **Jianjun Li:** resources, Validation. **Xin Wu:** supervision. **Xiaoqing Li:** Software, resources. **Changfeng Zhu:** Software, resources. **Jiamei Huang:** Validation. **Shuo Zhang:** Software. **Yulong Tang:** Conceptualization, Writing- Reviewing and Editing, Funding acquisition. **Yulong Yin:** Project administration, Methodology.

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.

Acknowledgments

This work was financially supported by the Innovation Team of Key areas of the Ministry of Science and Technology, Science and Technology Leadership Program of Hunan Province (2019RS3020); Special Fund for Key Program of Science and Technology of Yunnan Province (202102AE090046). Data availability: All data generated or analyzed during this study are included in this published article.

References

Allard J, Bucher S, Massart J, Ferron PJ, Le Guillou D, Loyant R, Daniel Y, Launay Y, Buron N, Begriche K, Borgne-Sanchez A, Fromenty B. Drug-induced hepatic steatosis in absence of severe mitochondrial dysfunction in HepaRG cells: proof of multiple mechanism-based toxicity. Cell Biol Toxicol 2021;37:151–75.

Aly FM, Othman A, Haridy MAM. Protective effects of fullerene C60 nanoparticles and virgin olive oil against genotoxicity induced by cyclophosphamide in rats. Oxid Med Cell Longev 2018;2018:1261356.

Baghban Kanani P, Daneshyar M, Aliakbarlu J, Hamian F. Effect of dietary turmeric and cinnamon powders on meat quality and lipid peroxidation of broiler chicken under heat stress condition. Vet Res Forum 2017;8:163–9.

Dias MM, Souza FR, Takada L, Feitosa FL, Costa RB, Diaz ID, Cardoso DF, Tonussi RL, Baldi F, Albuquerque LG, Oliveira HN. Study of lipid metabolism-related genes as candidate genes of sexual precocity in Nellore cattle. Genet Mol Res 2015;14:234–43.

Díazgarcía CM, Mongeon R, Lahmann C, Koveal D, Zucker H, Yellen G. Neuronal stimulation triggers neuronal glycolysis and not lactate uptake. Cell Metabol 2017;26(2):361.

Guo J, Shan T, Wu T, Zhu LN, Ren Y, An S, Wang Y. Comparisons of different muscle metabolic enzymes and muscle fiber types in Jinhua and Landrace pigs. J Anim Sci 2011;89:185–91.

Han F, Li J, Zhao R, Liu L, Li L, Li Q, He J, Liu N. Identification and co-expression analysis of long noncoding RNAs and mRNAs involved in the deposition of intramuscular fat in Aohan fine-wool sheep. BMC Genom 2021;22:98.

Hao T, Zhou J, Lu S, Yang B, Wang Y, Fang W, Jiang X, Lin Q, Li J, Wang C. Fullerene mediates proliferation and cardiomyogenic differentiation of adipose-derived stem cells via modulation of MAPK pathway and cardiac protein expression. Int J Nanomed 2016;11:269–83.

Hua ZG, Xiong LJ, Yan C, Wei DH, YingPai Z, Qing ZY, Lin QZ, Fei FR, Ling WY, Ren MZ. Glucose and insulin stimulate lipogenesis in porcine adipocytes: dissimilar and identical regulation pathway for key transcription factors. Mol Cell 2016;39:797–806.

Humam AM, Loh TC, Foo HL, Izuddin WI, Awad EA, Idrus Z, Samsudin AA, Mustapha NM. Dietary supplementation of postbiotics mitigates adverse impacts of heat stress on antioxidant enzyme activity, total antioxidant, lipid peroxidation, physiological stress indicators, lipid profile and meat quality in broilers. Animals (Basel) 2020;10.

Joo ST, Kim GD, Hwang YH, Ryu YC. Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Sci 2013;95:828–36.

Kim GD, Jeong JY, Jung EY, Yang HS, Lim HT, Joo ST. The influence of fiber size distribution of type iib on carcass traits and meat quality in pigs. Meat Sci 2013;94:267–73.

Lefaucheur L, Ecolan P, Plantard L, Gueguen N. New insights into muscle fiber types in the pig. J Histochem Cytochem 2002;50:719–30.

Li Y, Xu Z, Li H, Xiong Y, Zuo B. Differential transcriptional analysis between red and white skeletal muscle of Chinese Meishan pigs. Int J Biol Sci 2010;6:350–60.

Li YH, Li FN, Duan YH, Guo QP, Wen CY, Wang WL, Huang XG, Yin YL. Low-protein diet improves meat quality of growing and finishing pigs through changing lipid metabolism, fiber characteristics, and free amino acid profile of the muscle. J Anim Sci 2018;96:3221–32.

Liao S, Liu G, Tan B, Qi M, Li J, Li X, Zhu C, Huang J, Yin Y, Tang Y. Fullerene C60 protects against intestinal injury from deoxynivalenol toxicity by improving antioxidant capacity. Life (Basel) 2021;11.

Liao S, Tang S, Chang M, Qi M, Li J, Tan B, Gao Q, Zhang S, Li X, Yin Y, Sun P, Tang Y. Chloroquine downregulation of intestinal autophagy to alleviate biological stress in early-weaned piglets. Animals (Basel) 2020;10.

Liu X, Trakooljul N, Hadlich F, Murani E, Wimmers K, Ponsuksili S. MicroRNA-mRNA regulatory networking fine-tunes the porcine muscle fiber type, muscular mitochondrial respiratory and metabolic enzyme activities. BMC Genom 2016;17:531.

Nakagawa A, Aoyagi S, Omachi H, Ishino K, Nishino M, Rio J, Ewels C, Shinohara H. Isolation and structure determination of missing fullerenes Gd@C74(CF3) n through in situ trifluoromethylation. R Soc Open Sci 2018;5:181015.

Nozdrenko D, Matvienko T, Vygovska O, Bogutska K, Motuziuk O, Nurishchenko N, Prylutskyy Y, Scharff P, Ritter U. Protective effect of water-soluble C60 fullerene nanoparticles on the ischemia-reperfusion injury of the muscle soleus in rats. Int J Mol Sci 2021;22.

Rehfeldt C, Tuchscherer A, Hartung M, Kuhn G. A second look at the influence of birth weight on carcass and meat quality in pigs. Meat Sci 2008;78:170–5.

Rajamani K, Thirugnanasambandan SS, Natesan C, Subramaniam S, Thangavel B, Aravindan N. Squalene deters drivers of RCC disease progression beyond VHL status. Cell Biol Toxicol 2021;37:611–31.

Sharoyko VV, Shemchuk OS, Meshcheriakov AA, Vasina LV, Iamalova NR, Luttsev MD, Ivanova DA, Petrov AV, Maystrenko DN, Molchanov OE, Semenov KN. Biocompatibility, antioxidant activity and collagen photoprotection properties of C60 fullerene adduct with L-methionine. Nanomedicine 2021:102500.

Stachowiak M, Szydlowski M, Flisikowski K, Flisikowska T, Bartz M, Schnieke A, Switonski M. Polymorphism in 3' untranslated region of the pig PPARA gene influences its transcript level and is associated with adipose tissue accumulation. J Anim Sci 2014;92:2363–71.

Tang Y, Liao S, Liu G, Xiong X, Liu H, Li F, Tan Z, Kong X, Yin Y, Tan B. Advanced single-cell pooled CRISPR screening identifies C19orf53 required for cell proliferation based on mTORC1 regulators. Cell Biol Toxicol 2022;38:43–68.

Vereshchaka IV, Bulgakova NV, Maznychenko AV, Gonchar OO, Prylutskyy YI, Ritter U, Moska W, Tomiak T, Nozdrenko DM, Mishchenko IV, Kostyukov AI. C60 fullerenes diminish muscle fatigue in rats comparable to N-acetylcysteine or beta-alanine. Front Physiol 2018;9:517.

Wang X, Xue C, Wang X, Liu H, Xu Y, Zhao R, Jiang Z, Dodson MV, Chen J. Differential display of expressed genes reveals a novel function of SFRS18 in regulation of intramuscular fat deposition. Int J Biol Sci 2009;5:28–33.

Xu D, Wang Y, Jiao N, Qiu K, Zhang X, Wang L, Wang L, Yin J. The coordination of dietary valine and isoleucine on water holding capacity, pH value and protein solubility of fresh meat in finishing pigs. Meat Sci 2020;163:108074.

Xu Z, Chen W, Wang L, Zhou Y, Nong Q, Valencak TG, Wang Y, Xie J, Shan T. Cold exposure affects lipid metabolism, fatty acids composition and transcription in pig skeletal muscle. Front Physiol 2021;12:748801.

Zhang C, Luo J, Yu B, Zheng P, Huang Z, Mao X, He J, Yu J, Chen J, Chen D. Dietary resveratrol supplementation improves meat quality of finishing pigs through changing muscle fiber characteristics and antioxidative status. Meat Sci 2015a;102:15–21.

Zhang C, Luo JQ, Zheng P, Yu B, Huang ZQ, Mao XB, He J, Yu J, Chen JL, Chen DW. Differential expression of lipid metabolism-related genes and myosin heavy chain isoform genes in pig muscle tissue leading to different meat quality. Animal 2015b;9:1073–80.

Zhong Y , Yan ZM, Song B, Zheng C, Li F. Dietary supplementation with betaine or glycine improves the carcass trait, meat quality and lipid metabolism of finishing mini-pigs. Anim Nutr 2021;7:376–83.

Zhou Y, Ruan Z, Li XL, Mi SM, Jiang M, Liu WH, Yang HS, Wu X, Jiang GL, Yin YL. Eucommia ulmoides Oliver leaf polyphenol supplementation improves meat quality and regulates myofiber type in finishing pigs. J Anim Sci 2016;94:164–8.

Zhou Z, Lenk RP, Dellinger A, Wilson SR, Sadler R, Kepley CL. Liposomal formulation of amphiphilic fullerene antioxidants. Bioconjugate Chem 2010;21:1656–61.