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Effects of early-life exposure to topsoil on the muscle fiber characteristics and gene expression of weaned piglets

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Abstract

The objective of this experiment was to investigate the influence of early exposure to topsoil on the muscle fiber characteristics and transcription related myogenesis, intramuscular fat metabolism, muscle fiber types, and mTOR signaling pathway of weaned pigs. A Total of 180 piglets were separately assigned to No soil, Antibacterial soil, and Normal soil group (each group, n=60), and were fed ad libitum with common antibiotic-free corn-soybean meal diets until day-31. Ten pigs from each group with similar body weight were selected to be slaughtered, and the longissimus dorsi (LD) muscle samples were collected for histological analysis and measurements of genes and proteins expression levels. In the present study, the muscle fiber diameter and the area of Normal soil and Antibacterial soil group were significantly higher than No soil group ($P < 0.05$). The Normal soil significantly upregulated the gene expression of MyoG compared to No soil and Antibacterial soil groups ($P < 0.05$). The gene expression of CD36 and CPT-1 of Normal soil group was significantly lower than No soil group ($P < 0.05$), while HSL expression of Normal soil group was significantly higher than Antibacterial and No

soil groups ($P < 0.05$). The MyHC I of Normal soil group was significantly higher than No soil group ($P < 0.05$), but the expression MyHC IIa was lower than No soil group ($P < 0.05$). The protein expression expressed the similar result with gene expression. In addition, the Normal soil significantly increased the AMPK and mTOR phosphorylation compared to No soil and Antibacterial soil groups ($P < 0.05$). These data suggest that early exposure to topsoil regulates the muscle fiber growth, modulates the expression pattern related to myogenesis, muscle fiber type, intramuscular fat metabolism, and increases the phosphorylation of mTOR and AMPK pathways.

Introduction

In the modern pig industry, fewer than 6 % of the pig raised in the United States are housed in pasture of dirt pens, and most of them have been moved from outdoors into the slatted-floor indoor systems ¹. This phenomenon was caused by the increased space requirements as well as a need for more complex management practices for outdoor systems compared to indoor systems ². The pigs fed indoor rarely have the opportunity to touch the soil, which is a complex system consists of air, water, minerals, organic matter, and biota ³. The effects of this environmental change are still understudied for the fact that the outdoor system is not a feasible means of swine production for the majority pig industry. However, previous studies show that feeding piglets outdoors during lactation improves growth performance, carcass weight, and feed efficiency ^{4,5}. In addition, it was reported that early exposure to agricultural soil modulates the gut microbiota maturation of pigs during their early life ⁶. Despite these discoveries, many questions remain in

regard to the roles of the exposure to outdoor soil in regulating myogenesis, muscle fiber type, intramuscular fat metabolism.

A previous investigation confirmed that the muscle fiber characteristics could be affected by the animal rearing environment⁷. The muscle fiber characteristics (diameter, number, area) and fiber type composition are closely related to each other⁸, and it is a reflection of animal growth rate⁹. Usually, muscle fiber types are defined by the isoforms of myosin heavy chain (MHC) as MyHC I, IIa, IIb, and IIx, based on their different ATPase types¹⁰. The gene expression related to the intramuscular fat metabolism, such as PPAR γ , CPT1, SREBP1, etc., was affected by rearing systems¹¹. Different rearing environments may also impact the lipid, protein, and energy metabolism in skeletal muscle by modulating the mechanistic target of the rapamycin (mTOR) signaling pathway¹².

The objective of this experiment was to explore the effects of exposure to topsoil during pre-weaning on the muscle fiber characteristics and expression patterns of genes and proteins related to myogenesis, muscle fiber type, intramuscular fat metabolism, and mTOR pathway.

Results

Muscle fiber characteristics

The histochemical section of LD muscle is shown in Figure 1. The muscle fiber characteristics consisted of muscle fiber diameter, fiber area, and the number of fibers are shown in Table 2.

The muscle fiber diameter of Antibacterial soil group was significantly higher than Normal and No soil groups ($P < 0.05$), and the Normal soil group had a significantly higher diameter than the

No soil group ($P < 0.05$). The muscle fiber area of Antibacterial soil and Normal soil groups was significantly greater than No soil group ($P < 0.05$). However, there were no significant differences in muscle fiber number among each group ($P > 0.05$).

Myogenesis relative gene expression

The gene expression related to myogenesis (MyoG, Myf5, and MSTN) in the LD muscle was measured by RT-PCR is presented in Figure 2. The Normal soil significantly upregulated the gene expression of MyoG of LD muscle compared to No soil and Antibacterial soil groups ($P < 0.05$). However, there was no significant gene expression differences of Myf5 and MSTN between each group ($P > 0.05$).

Intramuscular fat relative gene expression

The gene expression related with intramuscular fat deposition and lipolysis are shown in Figure 3 and Figure 4. For fat deposition, the gene expression of CD36 in No soil group was significantly higher than Normal soil group ($P < 0.05$), but there was no significant difference between No soil group and Antibacterial soil group ($P > 0.05$). There were no significant differences of gene expression of PPAR γ , FAS, and SREBP-1 between each group ($P > 0.05$). The gene expression related with lipolysis such as HSL was significantly upregulated in Normal soil group compared to Antibacterial soil and No soil group ($P < 0.05$). The LPL expression of Normal soil group was significantly higher than No soil group ($P < 0.05$), yet no significant difference compared with Antibacterial soil group ($P > 0.05$) was noticed. However, the CPT1

was down regulated in Normal soil group compared with No soil group ($P < 0.05$). Additionally, there was no significant difference of ATGL expression between each group ($P > 0.05$).

Muscle fiber type relative gene expression

The expression levels of MyHC I, MyHC IIa, MyHC IIb and MyHC IIx were illustrated in Figure 5. The MyHC I of Normal soil group was significantly higher than No soil group ($P < 0.05$), but no significant difference compared to Antibacterial soil group ($P > 0.05$). However, the MyHC IIa of Normal soil group was significantly down regulated compared with No soil group ($P < 0.05$). There were no significant differences of MyHC IIb and IIx between each group ($P > 0.05$).

Protein expression related with intramuscular fat and myogenesis

HSL, PPAR γ , Myf5, and MSTN expressions were further measured using Western blot, and the results were displayed in Figure 6. The protein abundance of HSL in Normal soil group was significantly higher than Antibacterial soil and No soil group ($P < 0.05$). In addition, PPAR γ and MSTN protein abundances were significantly decreased in Normal soil group compared with the other two groups ($P < 0.05$). These protein expressions were consistent with the PCR results. However, there was no significant difference of Mfy5 abundance between each group ($P > 0.05$).

Muscle fiber type protein expression

The protein abundances of MyHC I, MyHC IIa, MyHC IIb and MyHC IIx were determined via Western blot (Figure 7). The protein expression of MyHC I and MyHC IIa was comparable with the PCR results. The MyHC I of Normal soil group was significantly greater than No soil and Antibacterial soil group ($P < 0.05$), and the MyHC IIa of Normal soil group was significantly lessened ($P < 0.05$). The MyHC IIx protein abundance in Normal soil group was also significantly decreased compared with other two groups ($P < 0.05$). Consistently, the MyHC IIb protein abundance was not significantly different among all groups ($P > 0.05$).

Protein expression related with mTOR pathway

AMPK and mTOR signaling pathways were determined using Western blotting analysis, and the outcomes were demonstrated in Figure 8. The results showed that the Normal soil significantly increased the AMPK and mTOR phosphorylation compared to No soil and Antibacterial soil groups ($P < 0.05$), and no significant differences were obvious between No soil and Antibacterial soil groups ($P > 0.05$).

Discussion

In relation to previous studies, muscle fiber characteristics (muscle fiber diameter, number, cross section area, and muscle fiber type) were affected by intrinsic factors (breed, sex, and age) and extrinsic factors such as nutrition value¹³⁻¹⁵. Based on the results, our study indicated that different topsoil treatments also had effects on the muscle fiber characteristics and the transcription related with myogenesis and muscle fiber type. Larzul, et al.⁹ pointed out that the muscle fiber cross section area had a positive relationship with the pig growth rate. Relevantly,

the muscle fiber area of Normal soil group and Antibacterial soil group was significantly higher than No soil group and indicated a better muscle growth rate. In addition, the muscle fiber diameter is positively related to the cross-section area¹³, and our results were consistent with this theory.

Hyperplasia (increasing muscle fiber number) and hypertrophy (increased fiber size) are two main processes that regulate muscle growth¹⁶. MRFs are a family of helix-loop-helix transcription aspects, including MyoG, Myf5, etc., that modify muscle hyperplasia and hypertrophy¹⁷. Myf5 is the primary MRFs which directly proliferates myogenic progenitor cells towards a myogenic lineage, and MyoG is the secondary MRFs which regulates the differentiation and fusion of myoblasts to form myofibers^{18,19}. The expression of MSTN is mainly in skeletal muscle and its expression has a negative relationship with muscle growth²⁰. It was also reported that the mutation of MSTN increased the muscle growth and muscle mass in many animals including pigs, sheep, rabbits, and cattle²¹⁻²⁴. This experiment confirmed that exposure to topsoil during the pre-weaning stage changed the transcription related with myogenesis. Exposure to Normal soil upregulated the gene expression of Myf5, while downregulated the MSTN transporter. The results indicated that exposure to topsoil had a positive impact on the myogenesis of piglets.

According to our results, the transcription of different muscle fiber types of weaned pigs was also affected by the topsoil. In general, there are four different muscle fiber types (type I, type IIa, type IIb, and type IIx) which can be detected in pig skeletal muscle, and they are distinguished by different types of myosin heavy chain (MyHC I, IIa, IIb, and IIx)¹⁰. Type I is slow-oxidative fiber, type IIa is fast oxide-glycolytic fiber, and both type IIb and IIx are fast glycolytic fibers²⁵. Different muscle fiber types represent different ATPase characteristics of

fibers ²⁶, for example, type I fiber is rich in mitochondria which provides ATP by its fatty acid oxidation, and type II fibers, which are classified as IIa, IIb, or IIx by its myosin heavy chain (MyHC) isoforms expression, utilize glucose to supply energy ^{10,27}. The muscle fiber types are impacted by complicated intrinsic and extrinsic factors such as breed, gender, age, nutrient level, and physical activity ²⁸. Based on this theory and our transcription results, exposure to the topsoil, as an extrinsic factor, also altered the muscle fiber type composition and the energy utilization forms of weaned pigs, and contacting with Normal soil increased the proportion of type I fiber which relies on the oxidative activity while decreased the type II fibers which have more glycolytic metabolism. The changes of muscle fiber characteristics of weaned pigs may also affect the postmortem metabolism or even the consequential meat quality.

In addition, the gene and protein expression related with intramuscular fat deposition and fat removal of piglets were influenced by the exposure to topsoil. For the intramuscular fat deposition, exposure to Normal soil downregulated the CD36 gene and PPAR γ protein expression. CD36 is a primary fatty acid transporter expressed in animal skeletal muscle ^{29,30}, and its abundance on the plasma membrane of obesity animals has a positive correlation with the rate of fatty acid uptake ³¹. PPAR γ is a ligand-activated transcription factor, expressed in many tissues (skeletal muscle and adipocytes) which accelerates adipogenesis and insulin sensitivity ^{32,33}, and its expression regulates the stimulation of adipocyte differentiation and fat deposition ³⁴. In terms of lipolysis, Normal soil unregulated the HSL, LPL expression, while downregulated the CPT1 gene expression. HSL detached fatty acids from intracellular triacylglycerol for oxidation and exportation ³⁵. LPL is a rate-limiting enzyme for the hydrolysis of triacylglycerol, and its catalyzed reaction products, fatty and monoacylglycerol, are used by adipose tissue and

skeletal muscle as element of neutral lipids ³⁶. CPT1 was reported to be correlated with mitochondrial fatty acid oxidation ³⁷.

The mTOR pathway plays an important role in modulating amino acid metabolism and reflecting the availability of amino acid ^{38,39}. In addition, AMPK is a key energy sensor which regulates the cellular energy ⁴⁰, and it can also modulates oxidative stress and mitochondrial function ⁴¹. In this investigation, we found that exposure to topsoil increased the mTOR and AMPK signaling pathway in weaned pigs, and it activated their phosphorylation. The results indicated that the amino acid and cellular energy metabolism of piglets were modulated by the exposure to topsoil during weaning period.

The current experiment proved that exposure to topsoil promotes the myogenesis, modulates the transcription related with muscle fiber types, intramuscular fat deposition and lipolysis, regulates the AMPK and mTOR signaling pathway of weaned piglets.

Material and methods

Animals and experimental design

The University of Arkansas's Institutional Animal Care and Use Committee approved all experimental procedures involving animals during the study (ethical approval code: 18059). This study followed the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (PLoS Bio 8(6), e1000412,2010). All experiments were performed in accordance with relevant guidelines and regulations. All 30 sows used in the study were blocked by parity and farrowing body weight. Piglets from each litter were cross-fostered within 24 hours across three sows (within similar parity and body weight). Six piglets with similar body weight from each litter

(n=180) were individually transferred to the same pen in the nursery facility, and kept in their littermates for the entire trial. Piglets were equally allocated to the No soil group, Antibacterial soil group, and Normal soil group (60 piglets in each group). No soil group was exposed to an empty pan, Antibacterial soil group was exposed to a pan with 1 kg of irradiated topsoil (Sterigenics, Fort Worth, TX) to kill bacteria in the soil, and Normal soil group was exposed to a pan with 1 kg of topsoil (Sod Store, Inc., Tontitown, AR). They were fed *ad libitum* with common antibiotic-free corn-soybean meal diets in littermates for 31 days.

Animal slaughter and sampling

The day prior to harvest, all pigs were weighed, and the pigs of median weight from each pen (10 piglets per group) were selected for sampling on day 31. Following a 12 h period of fasting prior to slaughter with the access of water, the pigs were transported to the University of Arkansas red meat abattoir. Piglets were euthanized by a captive bolt and immediately followed by exsanguination. Then, the muscle samples from *longissimus dorsi* (LD) of left carcass were removed and subsequently snap frozen at -80°C in liquid nitrogen for RNA isolation and protein extraction process. Another piece of LD muscle from each pig was cut into 0.5 × 0.5 × 1.0 cm cube, and immediately fixed in 10% buffered neutral formalin solution for the histological experiment.

Histological analysis

The LD muscle samples, fixed in 10% buffered neutral formalin solution, were dehydrated in alcohol, cleared in xylene, infiltrated, embedded in paraffin⁴², and then were cut in to 3 μm thickness. H&E (hematoxylin and eosin) staining was used to treat the thickness for histological study⁴³. Stained cross-sections were viewed and photographed at 175 × by ZOE™ Fluorescent

217 Cell Imager (Bio-rad, Hercules, CA, USA). Five photographs of each cross-section of LD
218 samples were taken, and then they were analyzed using Image-J software (National Institutes of
219 Health, Bethesda, MD, USA). The average muscle fiber numbers per area were obtained by
220 counting the total number of fibers in five areas ($700,000 \mu\text{m}^2$ for each area). The muscle fiber
221 diameter (μm) and area (μm^2) of each LD muscle was measured by using 300 fibers in five
222 cross-sections of each sample.

223 **RNA isolation and cDNA synthesis**

224 The samples, stored in liquid nitrogen, were homogenized using the Precellys Evolution
225 homogenizer (Bertin Technologies, Rockville, MD, USA) with TRIzolTM Reagent (Thermo
226 Fisher Scientific, Cat. No. 15596026). After homogenization, Direct-ZolTM RNA Miniprep Kit
227 (Zymo Research, Cat. No. R2072) was used to extract the total RNA from each samples of LD
228 muscle. To eliminate genomic DNA contamination, resultant total RNA was then treated with
229 DNase I, RNase-free (Promega, Madison, WI, USA), in accordance with manufacturer's
230 instructions. The RNA concentration was assessed by NanoDrop (Agilent Technologies, Santa
231 Clara, CA, USA). Finally, the purified RNA samples were subjected to reverse-transcription
232 using iScriptTM Cdna Synthesis Kit (Bio-rad, Cat. No. 1708890) following the instructions
233 given.

234 **Real-time qPCR**

235 According to the recorded sequences showed in GenBank, the primers of β -actin, MyoG, Myf5,
236 MSTN, PPAR- γ , FAS, SREBP-1, CD36, CPT-1, ATGL, LPL, HSL, MyHC I, MyHC IIa, MyHC
237 IIb, MyHC IIx were designed using GenBank for pig genes (Table 1). β -actin was chosen as the
238 house-keeping gene to normalize target gene levels. Real-time qPCR was performed by using

iQTM SYBR[®] Green Supermix (Bio-rad, Cat. No. 1708890) along with the manufacturer's instructions. The 2^{-ΔΔCt} method was used to analyze the relative expression, and the relative expression was normalized and expressed as a ratio to the expression in the No soil group.

Western Blot

Total proteins of LD muscle samples were extracted using T-PERTM Tissue Protein Extraction Reagent (Thermo Fisher Scientific, Cat. No. 78510) according to the guidelines. The concentration of protein samples was measured by Micro BCATM Protein Assay Kit (Thermo Fisher Scientific, Cat. No. 23235). Protein samples were separated on 7.5% Mini-PROTEAN[®] TGXTM Precast Protein Gels, 15-well, 15 μl (Bio-rad, Cat. No. 4561026), and then transferred onto Trans-Blot Turbo Midi Nitrocellulose Transfer Packs (Bio-rad, Cat. No. 1704159). Non-specific antibodies were excluded by blocking the membrane with 5% BSA (Bovine Serum Albumin) as blocking buffer for 120 min at 25°C. The membranes were incubated with primary antibodies over night at 4°C after blocking, and then were rinsed and incubated with secondary antibodies for 60 min at 25°C. The antibodies, including PPAR γ , HSL, Myf5, MSTN, MyHC I, MyHC IIa, MyHC IIb, MyHC IIx, p-AMPK, AMPK, p-mTOR, mTOR, GAPDH and HRP-conjugated anti-rabbit Ab were purchased from ABclonal Technology Inc. (Woburn, MA, USA). The ClarityTM Western ECL Substrate (Bio-rad, Cat. No. 1705060) was used to visualize bands, and then the bands were detected by ChemiDocTM Touch Gel Imaging System (Bio-rad, Hercules, CA, USA). The density of bands was normalized according to GAPDH content. The expression levels of different proteins were quantified using Image Lab Software (Bio-rad, Hercules, CA, USA) ⁴⁴.

Statistical analysis

All data were analyzed by one-way ANOVA with SPSS 22.0 statistical software (SPSS Inc., Chicago, IL, USA). The results were expressed as the Mean $\pm$ SEM, and a $P < 0.05$ was used to determine statistical significance.

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Contributions

Y.H., T.T., C.M., and J.Z. conceived and designed the experiment; Y.W., P.M., and S.Z. performed the experiments; Y.W. analyzed the data; Y.W. and Y.H. wrote and revised the paper. All authors have read and approved the final version of the manuscript.

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375 Correspondence to Yan Huang

376 **Competing interests**

377 The authors claim no competing interestes

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382 **Abbreviations**

383 AMPK: AMP-activated protein kinase

384 ATGL: adipose triglyceride lipase

385 CD36: cluster of differentiation molecule

386 CPT-1: carnitine palmitoyltransferase 1

387 FAS: Fas cell surface death receptor

388 H&E: hematoxylin and eosin

389 HSL: hormone-sensitive lipase

390 LD: longissimus dorsi

391 LPL: lipoprotein lipase

392 mTOR: mammalian target of rapamycin

393 MSTN: myostatin

394 MyHC-I: myosin heavy chain I

395 MyHC-IIa: myosin heavy chain IIa

396 MyHC-IIb: myosin heavy chain IIb
397 MyHC-IIx: myosin heavy chain IIx
398 Myf5: myogenic factor 5
399 MyoG: myogenin
400 PPAR- γ : peroxisome proliferator activated receptor gamma
401 SREBP-1: sterol regulatory element binding transcription factor 1

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403

404 (A) (B) (C)

405

406 Figure 1. Histological cross-section of *Longissimus dorsi* muscles in piglets. (A) No soil; (B)
407 Antibacterial soil; (C) Normal soil. Bar on lower right corner of each panel = 100 μ m.

408

Figure 2. Effect of topsoil on gene expression related with myogenesis of weaned piglets. Values are the least square mean $\pm$ standard error of the mean. Within a row, means with no superscripts or with a common superscript letter are not significantly different ($P < 0.05$).

414

415

416 Figure 3. Effect of topsoil on gene expression related with intramuscular fat deposition in
 417 weaned piglets. Values are the least square mean $\pm$ standard error of the mean. Within a row,
 418 means with no superscripts or with a common superscript letter are not significantly different (P
 419 < 0.05).

420

Figure 4. Effect of topsoil on gene expression related with intramuscular fat lipolysis in weaned piglets. Values are the least square mean $\pm$ standard error of the mean. Within a row, means with no superscripts or with a common superscript letter are not significantly different ($P < 0.05$).

Figure 5. Effect of topsoil on gene expression related with muscle fiber type of weaned piglets.

Values are the least square mean $\pm$ standard error of the mean. Within a row, means with no superscripts or with a common superscript letter are not significantly different ($P < 0.05$).

Figure 6. Effect of topsoil on protein expression related with intramuscular fat and myogenesis of weaned piglets. Values are the least square mean $\pm$ standard error of the mean. Within a row, means with no superscripts or with a common superscript letter are not significantly different ($P < 0.05$).

Figure 7. Effect of topsoil on muscle fiber type protein expression of weaned piglets. Values are the least square mean $\pm$ standard error of the mean. Within a row, means with no superscripts or with a common superscript letter are not significantly different ($P < 0.05$).

Figure 8. Effect of topsoil on AMPK and mTOR signaling pathways in the muscle of weaned piglets. Values are the least square mean $\pm$ standard error of the mean. AMPK: AMP-activated protein kinase; mTOR: mammalian target of rapamycin. Within a row, means with no superscripts or with a common superscript letter are not significantly different ($P < 0.05$).

452 Table 1. Primers used in this study

Gene ¹	Direction	Primer sequence	GenBank Accession No.
β -actin	Forward	5'- GGATGCAGAAGGAGATCACG -3'	DQ845171
	Reverse	5'- ATCTGCTGGAAGGTGGACAG -3'	
MyoG	Forward	5'- TCT ATG ACG GGG AAA ACT AC -3'	NM_001012406
	Reverse	5'- TGG AGC CAG AGT GGT GTA TC -3'	
Myf5	Forward	5'-GAGGATATTTCCAGTAAGTGGT -3'	NM_001278775
	Reverse	5'- AGCACTGCTAGCTTTCTCGG -3'	
MSTN	Forward	5'- CTCCTGCTTGACACCGACTT -3'	NM_214435
	Reverse	5'- TGGGTAGCATGGGGACAGTA -3'	
PPAR- γ	Forward	5'- ACTCAAAGCAGCAGGAAAGGT -3'	NM_214379
	Reverse	5'- TGTCACAAACTCACCTTAGGCT -3'	
FAS	Forward	5'- CACACTCCTGATCCGCACC -3'	NM_213839
	Reverse	5'- ACTCCCCATCACAGGGCTAT -3'	
SREBP-1	Forward	5'- TGCAGATAACACAAGCCGGT -3'	AY338729
	Reverse	5'- TGCTGCCCCGAGAGAAAAGAG -3'	
CD36	Forward	5'- GCCACTCCAAGGAGAACAGA -3'	NM_001044622
	Reverse	5'- GCAGGCCACAGTCTTCTACC -3'	
CPT-1	Forward	5'- CGGAAACGCCTTTTGGACAC -3'	AF284832
	Reverse	5'- GACTGGCGGAGGGAATACAG -3'	
ATGL	Forward	5'- CAAGACTCTGGGTAGCTGCG -3'	EU373817
	Reverse	5'- CTTTTCCCCCAGGACTCCAC -3'	
LPL	Forward	5'- CCAACGTGTCTGTTGTGGAT -3'	NM_214286
	Reverse	5'- CTGCTTCACCACCTTCTTGA -3'	
HSL	Forward	5'- CCAACGTGTCTGTTGTGGAT -3'	AY559451
	Reverse	5'- CTGCTTCACCACCTTCTTGA -3'	
MyHC-I	Forward	5'- CCAACGTGTCTGTTGTGGAT -3'	NM_213855

	Reverse	5'- CTGCTTCACCACCTTCTTGA -3'	
MyHC-IIa	Forward	5'- CCAACGTGTCTGTTGTGGAT -3'	NM_214136
	Reverse	5'- CTGCTTCACCACCTTCTTGA -3'	
MyHC-IIb	Forward	5'- CCAACGTGTCTGTTGTGGAT -3'	NM_001123141
	Reverse	5'- CTGCTTCACCACCTTCTTGA -3'	
MyHC-IIx	Forward	5'- CCAACGTGTCTGTTGTGGAT -3'	NM_001104951
	Reverse	5'- CTGCTTCACCACCTTCTTGA -3'	

MyoG: myogenin; Myf5: myogenic factor 5; MSTN: myostatin; PPAR- γ : peroxisome
 proliferator activated receptor gamma; FAS: Fas cell surface death receptor; SREBP-1: sterol
 regulatory element binding transcription factor 1; CD36: cluster of differentiation molecule;
 CPT-1: carnitine palmitoyltransferase 1; ATGL: adipose triglyceride lipase; LPL: lipoprotein
 lipase; HSL: hormone-sensitive lipase; MyHC-I: myosin heavy chain I; MyHC-IIa: myosin
 heavy chain IIa; MyHC-IIb: myosin heavy chain IIb; MyHC-IIx: myosin heavy chain IIx

Table 2. Effect of topsoil on muscle fiber characteristics of weaned piglets

Item	No soil	Antibacterial soil	Normal soil	<i>P</i> -value
Muscle fiber diameter / μm	45.02 ± 0.53^c	50.53 ± 0.69^a	48.75 ± 0.66^b	0.047
Muscle fiber area / μm^2	1703.24 ± 41.68^b	2188.73 ± 61.82^a	2037.1 ± 58.42^a	0.043
Muscle fiber number	229.91 ± 7.69	210.02 ± 8.04	216.75 ± 12.62	0.197

Values are the least square mean $\pm$ standard error of the mean. Within a row, means with no superscripts or with a common superscript letter are not significantly different ($P < 0.05$).

Supplementary Files

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- [SRSupplementaryfile.pdf](#)