Betaine affects muscle lipid metabolism via regulating the fatty acid uptake and oxidation in finishing pig

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Abstract

Background: Betaine affects fat metabolism in animals, but the specific mechanism is still not clear. The purpose of this study was to investigate possible mechanisms of betaine in altering lipid metabolism in muscle tissue in finishing pigs.

Methods: A total of 120 crossbred gilts (Landrace × Yorkshire × Duroc) with an average initial body weight of 70.1 kg were randomly allotted to three dietary treatments. The treatments included a corn–soybean meal basal diet supplemented with 0, 1250 or 2500 mg/kg betaine. The feeding experiment lasted 42 d.

Results: Betaine addition to the diet significantly increased the concentration of free fatty acids (FFA) in muscle (P < 0.05). Furthermore, the levels of serum cholesterol and high-density lipoprotein cholesterol were decreased (P < 0.05) and total cholesterol content was increased in muscle (P < 0.05) of betaine fed pigs. Experiments on genes involved in fatty acid transport showed that betaine increased expression of lipoprotein lipase (LPL), fatty acid translocase/cluster of differentiation (FAT/CD36), fatty acid binding protein (FABP3) and fatty acid transport protein (FATP1) (P < 0.05). The abundance of fatty acid transport protein and fatty acid binding protein were also increased by betaine (P < 0.05). As for the key factors involved in fatty acid oxidation, although betaine supplementation didn't affect the level of carnitine and malonyl-CoA, betaine increased mRNA and protein abundance of carnitine palmitransferase-1 (CPT1) and phosphorylated-AMPK (P < 0.05).

Conclusions: The results suggested that betaine may promoted muscle fatty acid uptake via up-regulating the genes related to fatty acid transporter including FAT/CD36, FATP1 and FABP3. On the other hand, betaine activated AMPK and up-regulated genes related to fatty acid oxidation including PPARα and CPT1. The underlying mechanism regulating fatty acid metabolism in pigs supplemented with betaine is associated with the up-regulation of genes involved in fatty acid transport and fatty acid oxidation.

Keywords: Betaine, Fatty acid intake, Fatty acid oxidation, Muscle, Pig

Background

Betaine is a derivative of the amino acid glycine with three chemically reactive methyl groups. Betaine is distributed widely in animals, plants and microorganisms, and it is also a metabolite of choline oxidation in animals [1]. The principal physiologic role of betaine is as a methyl group donor [2], which means betaine participates in many important biochemical pathways, including methionine-homocysteine cycle and the biosynthesis of many compounds such as carnitine, creatine and phospholipids. Since carnitine is required for transport of long chain fatty acids into mitochondria [3], scientists have paid much attention to effects of betaine on energy metabolism especially lipid metabolism in animals. Studies showed that dietary betaine supplementation affected energy partitioning in pigs [4, 5] and it's also widely reported that betaine promotes animal growth and decreases carcass fat percentage in finishing pigs [6–10]. Further investigations found that betaine supplementation could decrease hepatic triglyceride accumulation [11, 12] and prevent fatty liver in rats fed high-fat-diets.
[13, 14]. The intramuscular fat content in the longissimus muscle was increased when pigs were fed betaine [15, 16]. Madeira et al. [17] reported that betaine might be involved in the differential regulation of some key genes of lipid metabolism in muscle and subcutaneous adipose tissue. However, studies on the mechanism of betaine affecting lipid metabolism in muscle are lacking. Therefore, the objective of the present study was to investigate possible mechanisms of betaine in altering lipid metabolism in muscle tissue of finishing pigs.

**Methods**

**Animals and treatments**

The experiment protocol used in this study was approved by the Institutional Animal Care and Use Committee of Zhejiang University. A total of 120 crossbred gilts (Landrace × Yorkshire × Duroc) with an average initial body weight of 70.1 kg (SD 0.70 kg) were randomly allotted to three dietary treatments. Each treatment consisted of four pens replicates with 10 gilts per pen. The treatment diets included a corn–soybean meal basal (Table 1) supplemented with 0, 1250 mg/kg (Low Betaine) or 2500 mg/kg (High Betaine) betaine (provided by Healthy Husbandry Sci-tech Co., Ltd. Hangzhou, China) respectively at the expense of corn. The basal diet was formulated to meet or exceed the nutrient requirements of finishing pigs [18]. Chemical analyses of the basal diet were carried out according to the methods of AOAC [19]. The feeding experiment lasted 42 d after a 7-day adaptation period. All pigs were housed in a curtain-sided pig barn with concrete slotted floors. Feed and water were provided for ad libitum consumption throughout the experiment.

**Table 1** Nutrition formulation of basic diet

| Ingredients               | %    | Nutrient % |
|---------------------------|------|------------|
| Corn                      | 67.83| Digestible energy, M/kg a 13.42 |
| Soybean meal              | 23   | Dry matter 87.09 |
| Rapeseed meal             | 3    | Crude protein 17.02 |
| Wheat midding             | 3    | Crude fat 3.98 |
| Ca-HPO4                   | 1.5  | Calcium 0.85 |
| Limestone                 | 1.0  | Phosphorus 0.64 |
| Salt                      | 0.3  | Lysine 0.92 |
| Lysine                    | 0.10 | Met 0.27 |
| Trace element premix b    | 0.25 |            |
| Vitamin premix c          | 0.02 |            |

*a All of the data were analyzed value except digestible energy which was calculated using swine NRC(2012) values

*b Provided the following amounts per kilogram of diet: Fe (FeSO4·7H2O), 50 mg; Cu (CuSO4·5H2O), 5 mg; Mn (MnSO4·H2O), 5 mg; Zn (ZnSO4·7H2O), 50 mg; I (KI), 0.35 mg; Se (NaSe2O3), 0.15 mg

*c Provided the following amounts per kilogram of diet: vitamin A, 3000 IU; vitamin D3, 610 IU; vitamin E, 20 IU; vitamin B12, 0.021 mg; biotin, 0.1 mg; pantothenic acid, 10 mg; nicotinic acid, 15 mg

**Sample collection**

At the end of the trial, eighteen pigs (six from each dietary treatment) weighing about 111.8 kg (SD 2.08 kg) were selected to collect tissue samples. Following an overnight fast, pigs were stunned by electrical shock and bleeding. Individual blood samples were collected at slaughter during exsanguinations. After collection of blood, samples were kept at room temperature for 2 h and then centrifuged for 10 min at 3000×g at 4 °C. Serum was collected and frozen at ~80 °C until subsequent analyses. Samples of longissimus muscle between the 6th and 7th rib were obtained on the left side of the carcass within 5 min after slaughter, and then snap frozen in liquid nitrogen and stored at ~80 °C until subsequent analyses.

**Analysis of lipid metabolites in serum**

Serum concentration of high-density lipoprotein cholesterol (HDLC), total cholesterol (TC), free fatty acid (FFA) and triglyceride were measured with commercial assay kits (Nanjing Jiancheng Bio-engineering Institute, Code No. A112–2, A111–2, A042–1 and A110–2, respectively, Nanjing, China) following the manufacturer’s instructions.

**Muscle lipid metabolites analysis**

A 10% muscle homogenate was prepared with a mixture of chloroform and formaldehyde (a volume ratio of 2:1). Then extracted at room temperature for 24 h [20]. The organic solvent layer was taken and the level of triglyceride in muscle was measured with commercial assay kit (Nanjing Jiancheng Bio-engineering Institute, A110–2, Nanjing, China). Before the levels of TC and FFA in muscle were measured by the kits (Nanjing Jiancheng Bio-engineering Institute, Code No. A112–2, A111–2, A042–1 and A110–2, respectively, Nanjing, China), muscle tissue was made homogeneous with physiological saline. The concentrations of carnitine and malonyl-CoA were measured using ELISA kits (Biovol Technologies, Code No.50R–E.3088P & 50R–E.3035P, Shanghai, China) for porcine assay according to the instructions.

**RT-PCR analysis**

Total RNA was extracted from frozen porcine muscle tissue using the Trizol reagent as described by the manufacturer (Invitrogen). The RNA concentration and purity were determined by the NanoDrop ND-2000 spectrophotometer (Thermofisher, USA) and its integrity was confirmed by agarose gel electrophoresis. The cDNA synthesis was performed in a 10-μL reaction volume containing 2 μg total RNA using the SYBR Prime-Script™ RT-PCR kit with gDNA Eraser (Code No. RR047A, TaKaRa, Dalian, China). Genomic DNA is eliminated by treatment for 2 min at 42 °C with gDNA.
Eraser, which has potent DNA degrading activity. Then a reverse-transcription reaction reagent is added that includes a component that completely inhibits DNA degradation activity, and the reverse-transcription reaction proceeds for 15 min at 37 °C. The abundance of the target genes was measured by quantitative real-time PCR, performed with the ABI Stepone Plus™ RT-PCR system (ABI Biotechnology, USA) using SYBR Premix Ex Taq™ (Tli RNaseH Plus) RT-PCR kit (TaKaRa, Dalian, China). Primers for the selected genes were synthesized commercially by Invitrogen (Shanghai, China), shown in Table 2. The reaction protocol comprised a cycle of 95 °C for 1 min, 40 cycles of 95 °C for 10 s and 64 °C for 25 s. The expression of the target genes was normalized by the endogenous housekeeping gene (β-actin) [21, 22]. Each sample was analyzed in triplicate and the PCR amplification efficiency was close to 100%. The gene expression was calculated by using the comparative \(2^{-\Delta\Delta Ct}\) method [23].

**Western blot analysis**

Protein form muscle samples was extracted by T-PER Tissue Protein Extraction Reagent containing protease inhibitor cocktail (Thermo Pierce, Code No.78510, USA), and quantified with BCA protein assay kit (Beyotime, Code No.P0010, Shanghai, China) according to kit instructions. Proteins were separated on SDS - PAGE gels (12%), and then electrophoretically transferred onto immobilon-P polyvinylidene fluoride membranes (PVDF membrane, Millipore, Code No. IPVH00010, America). Membranes were blocked 1 h in Tris-buffered saline containing 5% nonfat-dried milk at room temperature. Membranes were then incubated overnight at 4 °C in blocking buffer containing primary antibodies (as shown in Table 3). A goat anti-rabbit IgG (H + L) Secondary antibody (Thermo Pierce, Code NO.31210, USA) with 1/5000 dilution was used in the detection of specific proteins. For loading control, β-actin antibody was used as control. In addition, the relative expression of p-AMPK was normalized with AMPK. Finally, Super Signal West Dura Extended Duration Substrate (Thermo Pierce, Code No. 34075, USA) was used to visualize the protein bands. Band intensities were determined by using BandScan 5.0 software.

The relative expressions of target proteins = \(\frac{\text{The optical density of target proteins}}{\text{The optical density of } \beta-\text{actin}}\).

**Statistical analyses**

Results were presented as means and standard deviations. Statistical analysis was performed by one-way analysis of variance (ANOVA) and the Duncan method was used to put up multiple comparison with the statistical software SPSS 19.0. In all analyses, the level of significant difference was set at \(P < 0.05\).

**Results**

**Betaine on serum lipid metabolites**

As shown in Fig. 1, there was no significant difference in the levels of serum FFA and triglyceride in the pigs fed

| Genes | GenBank accession | Primers sequences(5′ to 3′) | Product size, bp | Annealing temperature, °C |
|-------|-------------------|-----------------------------|-----------------|--------------------------|
| β-actin | XM_003124280.3 | CCTGCGGCTACCCAGGAAC TGTCCGCGATGCTTGGGTA | 123 | 63 |
| AMPKa2 | AY195788.1 | GGTGTGGTCTACCTACACCTCA GGCCTCTGCCAGTGACAAAT | 90 | 63 |
| PPARγ | NM_214379 | GGTGGAGCAGCAGCTGGTTG GGGAGGACTCTGGGTGGTTCA | 108 | 64 |
| LPL | NM_214286.1 | CCACTATAACAGAGGGGAACCCGAT CCGCCATCCAGTCAAACAGT | 138 | 64 |
| CPT1 | NM_001007191.1 | GGACAGAGAGCTGCCACCACCTATGAC TCTGAACGGCAGTGAGGTTGA | 128 | 63 |
| FATP1 | NM_001083931.1 | CCCTCTGGCAGCTCCTGTTGAT GCTGCGGTCCCCGAAATACA | 151 | 63 |
| FAT/CD36 | NM_001044622.1 | CTGTGTCCTGATCTTGAGCTG ACAAATGGTGAACTTCTCCACACTACACAC | 160 | 64 |
| FABP3 | NM_001099931.1 | CCAACAGTACAAAGCTCCCAACAC ACAAGTTTGGCCGCTCCATCTTCAGTGT | 176 | 63 |
| PPARα | NM_001044526.1 | GGTCTCATATCTGGCCAGAATGGA GAAGACCAAGAGGGGAACCCGAT CGCCTCAGCCTGACAAACAGT | 168 | 64 |
betaine compared with control group. Additionally, the concentration of HDLC and TC were significantly lower in the betaine treated pigs ($P < 0.05$).

**Betaine on muscle lipid metabolites**

The level of FFA and TC were markedly higher in muscle when pigs were fed betaine ($P < 0.05$, Fig. 2). Compared to the control group, the level of triglyceride in muscle was not affected by betaine addition ($P > 0.05$).

**Key factors involved in muscle FFA intake**

As shown in Fig. 3, the gene expression of FAT/CD36, FATP1 and PPARγ ($P < 0.05$) were higher in betaine-fed groups than control group. The addition of 2500 mg/kg betaine markedly up-regulated the gene expression of FABP3 and LPL ($P < 0.05$). In addition, the abundance of fatty acid transport protein and fatty acid binding protein were significantly increased by betaine supplementation ($P < 0.05$, Fig. 4).

**Key factors involved in muscle FA oxidation**

Betaine supplementation did not affect carnitine or malonyl-CoA in muscle compared to the control group ($P > 0.05$, Fig. 5).

The gene expression of AMPKa2, PPARα and CPT1 were significantly higher in pigs fed with betaine than the control group ($P < 0.05$, Fig. 6). Furthermore, betaine supplementation markedly increased the abundance of phosphorylated-AMPK and CPT1 in muscle ($P < 0.05$, Fig. 7).

**Discussion**

Fatty acid metabolism in muscle includes uptake, synthesis and oxidation [24–26], but the synthesis is at a slow rate [27]. The main source of fatty acid in muscle tissue includes transport from plasma and hydrolysis from chylomicron and very-low-density-lipoprotein (VLDL) with LPL. Our study found that the concentration of FFA was significantly increased in muscle when pigs

| Table 3 The primary antibodies for Western blot |
|-----------------------------------------------|
| **Primary antibody** | **Order numbers** | **Dilution** | **Size, kDa** |
|--------------------|------------------|-------------|--------------|
| Anti-Cardiac FABP  | abca ab45966     | 1:1500      | 15           |
| Anti-FATP1         | abcam ab81875    | 1:2000      | 65           |
| Anti-CPT1B         | abcam ab104662   | 1:2000      | 88           |
| Anti-Phospho-AMPK  | Cell Signaling Technology 2535 | 1:1000 | 62 |
| Anti-AMPKa         | Cell Signaling Technology 5932 | 1:1000 | 62 |
| β-actin (C4)       | Santa Cruz SC-47778 | 1:1500 | 43 |

![Fig. 1](image1.png)

**Fig. 1** Effect of betaine supplementation on serum parameters of lipid metabolism. The levels of serum free fatty acid (FFA, **a**), triglyceride (**b**), total cholesterol (**c**) and high-density lipoprotein cholesterol (HDLC, **d**) $^{a,b}$Values without common superscript letters differ significantly ($P < 0.05$). Low betaine and high betaine represent 1250 mg/kg and 2500 mg/kg betaine addition, respectively.
were fed betaine, similarly to the studies carried out by Yang et al. [28] and Fernández-Figares et al. [29]. We speculated that the transport of FFA and/or the hydrolysis may be enhanced. More experiments were carried out regarding factors involved in fatty acid transport in muscle tissue. It is widely recognized that long chain fatty acid (LCFA) cross the plasma membrane via a protein-mediated mechanism. A number of fatty acid transporters have been identified, including fatty acid translocase/cluster of differentiation (FAT/CD36) and fatty acid transport proteins (FATP1) [30]. We found that betaine supplementation up-regulated gene expression for

![Fig. 2](image) Effect of betaine supplementation on total cholesterol, FFA and triglyceride in muscle. The levels of total cholesterol (a), free fatty acid (FFA, b) and triglyceride (c) in muscle. Values without common superscript letters differ significantly (P < 0.05). Low betaine and high betaine represent 1250 mg/kg and 2500 mg/kg betaine addition, respectively.

![Fig. 3](image) The relative gene expression of key factors involved fatty acid uptake in muscle. mRNA expression was performed by RT-PCR and β-actin was chosen as reference gene. (a) The relative expression of FAT/CD36, FATP1, LPL and PPARγ in muscle. (b) The relative expression of FABP3 in muscle. Values without common superscript letters differ significantly (P < 0.05). Low betaine and high betaine represent 1250 mg/kg and 2500 mg/kg betaine addition, respectively.
FATP1 and FAT/CD36. Experiments in vitro have shown that over expression of FATP1 increased the uptake of LCFA in cells [31] and studies in vivo documented that muscle-specific over-expression of FAT/CD36 enhanced cellular fatty acid uptake in mice [32]. FABP3, another important protein in fatty acid transportation, plays a role in transporting fatty acid from the sarcolemma to their intracellular sites of metabolism [33]. In muscle cells, the intracellular transport of LCFAs is facilitated to a great extent by FABP3 [34] Additionally, FABP3 is confirmed to be associated with intramuscular fat in pigs [35]. Our studies showed that feeding betaine up-regulated the protein abundance of FABP3. In addition, the gene expression of FABP3 was enhanced when pigs were fed with 2500 mg/kg betaine but no difference was found with 1250 mg/kg betaine addition. The possible reason maybe that FABP3 expression is translationally rather than transcriptionally regulated [36]. In summary, betaine may promote the uptake of fatty acids in muscle via regulating the expression of FAT/CD36, FATP1 and FABP3. As mentioned above,
LPL is the principal enzyme that hydrolyzes circulating triglycerides and it also can increase lipid uptake [37]. The results showed a significant increase in the gene expression of LPL with the addition of 2500 mg/kg betaine, which indicates betaine might enhance lipid uptake as well as chylomicron hydrolysis. The nuclear receptor PPARγ is a central regulator of adipose tissue development and an important modulator of expression in adipocytes [38]. To date, only a limited number of genes are known to be direct targets of PPARγ in adipose tissue. The majority of these encode proteins with direct links to lipid metabolism including LPL, FATP and FAT/CD36 [39, 40]. In present study, the gene expression of PPARγ was significantly higher in betaine-fed groups than the control group. We found that the effect of betaine on PPARγ was similar to its downstream target genes. All these results were similar to Albuquerque [41] and imply that betaine may facilitate fatty acids uptake in muscle via affecting key factors involved in FFA uptake, and the specific regulation mechanism needs more research.

The concentration of FFA in muscle tissue results from the balance of transport and oxidation. As a methyl donor, betaine participates in the biosynthesis of carnitine and because of this, betaine may be related to...
fatty acid β-oxidation. LCFAs are first transformed into acyl CoA, then transferred into mitochondria after combining with carnitine where it is oxidized. Carnitine palmitoyl transferase I (CPT1) is the rate-limiting enzyme that controls the step of combination and malonyl-CoA is an allosteric inhibitor of CPT1 [42]. Whereas the synthesis of malonyl-CoA is catalyzed by acetyl-CoA carboxylase (ACC), the activity of the ACC is regulated by phosphorylation of AMPK [43]. Hence, AMPK-ACC-CPT1 is an important signaling pathway to regulate fatty acid β-oxidation in mitochondria. Cai et al. [44] found that gestational dietary betaine supplementation down-regulated expression of ACC in neonatal piglets and Pekkinen et al. [11] found betaine supplementation had an impact on carnitine metabolism in high-fat-fed mice. Our experiment didn’t find significant changes in muscle concentrations of malonyl-CoA or carnitine. The different results might be related to the different experiment condition and the mechanism needs to be further investigated. Increased gene expression and protein expression of CPT1 were up-regulated with betaine addition, which implied betaine may enhance fatty acid β-oxidation in muscle tissue. However, others have shown betaine supplementation reduced the activity of CPT1 and mRNA abundance, and further increased IMF in finishing pigs [Duroc × (Seghers × Seghers)] [15]. We speculate that the effect of betaine addition on CPT1 might be influenced by breed and muscle type. In order to get a better understanding, we further analyzed effects of betaine on AMP-activated protein kinase (AMPK) and PPARα, which are both upstream regulatory factors of CPT1. AMPK is a crucial energy sensor for cells, which can promote the catabolism of fatty acids by enhancing their uptake into mitochondria and their consequent breakdown by beta-oxidation [45]. It was reported that activated AMPK in muscle enhances the gene expression of PPARα and CPT1 [46], and CPT1 also seems to be a target of PPARα [47]. In the current experiment, the gene expression of both PPARα and AMPK were higher in betaine-fed groups as well as protein expression of p-AMPK (the activated form of AMPK). Similar to our previous report in rat liver [12], it can be inferred that betaine affected fatty acid oxidation in muscle via activating AMPK and up-regulated PPARα and CPT1 gene expression.

The effect of betaine supplementation on cholesterol metabolism was of interest. The present study showed that betaine supplementation decreased the concentration of serum cholesterol and HDLC and increased cholesterol level in muscle, which was consistent with the studies by Albuquerque et al. [41] and Yang et al. [20]. However, Matthews et al. [48] and Martins et al. [49] reported that betaine supplemented pigs presented higher serum cholesterol. The efficacy of betaine in regulating the concentration of cholesterol in pigs shows variable results and seems to depend on both animal and dietary factors. Although the results were inconsistent, it seems to indicate that betaine might affect cholesterol partitioning or maybe enhances the transport of cholesterol in pigs, and more research is needed to clarify the specific mechanism.

Conclusions
In present study, betaine supplementation increased the level of free fatty acids in muscle, which may have resulted due to a change in the balance of fatty acid uptake and oxidation. Betaine may promote fatty acid uptake via increasing the expression of fatty acid transporters including FAT/CD36, FATP1 and FABP3 in muscle. Additionally, betaine activated AMPK and up-regulated PPARα and CPT1, and may enhance fatty acid oxidation in muscle. Fatty acid accretion in muscle represents a balance between uptake and oxidation, and it seems that the effect of betaine on uptake was stronger than oxidation.

Abbreviations
CPT1: Carnitine palmitoyl transferase 1; FABP3: Fatty acid binding protein; FAT/CD36: Fatty acid translocase/cluster of differentiation; FATP1: Fatty acid transporter protein1; FFA: Free fatty acid; HDLC: High-density lipoprotein cholesterol; LPL: Lipoprotein lipase; TC: Total cholesterol

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Availability of data and materials
The raw data for the current study are available from the corresponding author on reasonable request.

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Authors’ contributions
YW and JF designed the study. SL and HW were involved in performing the experiment and data interpretation. SL drafted the manuscript and XW revised the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval
The experiment protocols used in this study was approved by the Institutional Animal Care and Use Committee of Zhejiang University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References
1. Eklund M, Bauer E, Wamatsu J, Mosenthin R. Potential nutritional and physiological functions of betaine in livestock. Nutr Res Rev. 2005; 18(01):31–48.
2. Craig SAS. Betaine in human nutrition. Am J Clin Nutr. 2004;80(3):539–49.
3. Wray-Cahen D, Fernández-Figares I, Vitanen E, Steele NC, Caperna TJ. Betaine improves growth, but does not induce whole body or hepatic palmitate oxidation in swine (Sus Scrofa Domestic). Comp Biochem Physiol A Mol Integr Physiol. 2004;137(1):131–40.

4. Fernández-Figares I, Wray-Cahen D, Steele NC, Campbell RG, Hall DD, Vitanen E, et al. Effect of dietary betaine on nutrient utilization and partitioning in the young growing feed-restricted pig. J Anim Sci. 2002;80(2):421–8.

5. Schrama JW, Heetkamp MJW, Simmins PH, Gerrits WJJ. Dietary betaine supplementation affects energy metabolism of pigs. J Anim Sci. 2003;81(5):1202–9.

6. Wang YZ, Xu ZR, Feng J. The effect of betaine and DL-methionine on growth performance and carcass characteristics in meat ducks. Anim Feed Sci Technol. 2004;116(1):151–9.

7. Feng J, Liu X, Wang YZ, Xu ZR. Effects of betaine on performance, carcass characteristics and hepatic betaine-homocysteine methyltransferase activity in finishing barrows. Asian Australas J Anim Sci. 2006;19(3):302–8.

8. Huang QC, Xu ZR, Han XY, Li WF. Changes in hormones, growth factor and lipid metabolism in finishing pigs fed betaine. Livest Sci. 2006;105(1):78–85.

9. Huang QC, Xu ZR, Han XY, Li WF. Effect of betaine on growth hormone pulsatile secretion and serum metabolites in finishing pigs. J Anim Physiol Anim Nutr (Berl). 2007;91(3–4):485–90.

10. Nakay J, Popova T, Vasilieva V. Influence of dietary betaine supplementation on the growth performance and carcass characteristics in male and female finishing-growing pigs. Bulg J Agric Sci. 2009;15(3):263–8.

11. Peukkala J, Olli K, Huotari A, Tiihonen K, Keski-Rahkonen P, Lehtonen M, et al. Betaine supplementation causes increase in carnitine metabolites in the muscle and liver of mice fed a high-fat diet as studied by nontargeted LC-MS metabolomics approach. Mol Nutr Food Res. 2013;57(1):159–68.

12. Xu L, Huang D, Wu Q, Wu J, Wang Y, Feng J. Betaine alleviates hepatic lipid accumulation via enhancing hepatic lipid export and fatty acid oxidation in rats fed with a high-fat diet. Br J Nutr. 2015;113(12):2235–43.

13. Zhang W, Wang L, Wang L, Li X, Zhang H, Luo J, et al. Betaine protects against high-fat-diet-induced liver injury by inhibition of high-mobility group box 1 and toll-like receptor 4 expression in rats. Dig Dis Sci. 2013;58(11):3198–206.

14. Demincie R, Da Silva RP, Lamarre SG, Kelly KB, Jacobs RL, Brossnan ME, et al. Betaine supplementation prevents fatty liver induced by a high-fat diet: effects on one-carbon metabolism. Amino Acids. 2015;47(4):839–46.

15. Liu Q, Han XY, Wang YZ, Yang XY, Chen T, Zheng XT. Betaine suppresses carnitine palmitoyltransferase I in skeletal muscle but not in liver of finishing pigs. Livest Sci. 2009;126(1):130–5.

16. Martins JM, Neves JA, Freitas A, Tirapicos J. Effect of long-term betaine supplementation on chemical and physical characteristics of three muscles from the Alentejano pig. J Sci Food Agric. 2012;92(10):2122–7.

17. Madeira MS, Rola EA, Afia OA, Pires VR, Lorton R, Doran O, et al. Influence of betaine and arginine supplementation of reduced protein diets on fatty acid composition and gene expression in the muscle and subcutaneous adipose tissue of cross-bred pigs. Br J Nutr. 2016;115(6):937–50.

18. National Research Council. Nutrient requirements of swine: eleventh revised edition. Washington, D.C.; The National Academies Press; 2012.

19. Association of Official Analytical Chemists (AOAC). Van Nostrand’s Encyclopedia of Chemistry. Hoboken: John Wiley & Sons, Inc. 2005.

20. Zeng T, Xie K, Zhang C, Yu L, Zhu Z. Determination the level of triglycerides in liver with chloroform/methanol homogenate. J Hyg Res. 2008;37(5):550–1. (In Chinese)

21. Nygaard A-B, Jørgensen CB, Cirea S, Fredholm K. Selection of reference genes for gene expression studies in pig tissues using SYBR green qPCR. BMC Mol Biol. 2007;8(1):67.

22. Everts V, Van Poucke M, Vandesompele J, Goossens K, Van Zeveren A, Hertzel AV, et al. Novel role of FATP1 in mitochondrial fatty acid oxidation in skeletal muscle cells. J Lipid Res. 2009;50(9):1789–99.

23. Ibrahimi A, Bonen A, Blinn WD, Hajri T, Ji X, Zhong K, et al. Muscle-specific overexpression of FAT/CD36 enhances fatty acid oxidation by contracting muscle, reduces plasma triglycerides and fatty acids, and increases plasma glucose and insulin. J Biol Chem. 1999;274(36):26716–61.

24. Hertzel AV, Bernhöf DA. The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. Trends Endocrinol Metab. 2001;12(1):75–80.

25. Glatt JFC, Schaaf GP, Binas B, Bonen A, Van Der Vusse GJ, Luiken JFP. Cytoplasmatic fatty acid-binding protein facilitates fatty acid utilization by skeletal muscle. Acta Physiol Scand. 2003;178(4):367–71.

26. Cho KH, Kim MJ, Jeon GJ, Chung HY. Association of genetic variants for FABP3 gene with back fat thickness and intramuscular fat content in pigs. Mol Biol Rep. 2011;38(3):2161–6.

27. Berghmans F, Verburg FJ, Van Moerkerk HTB, Engel B, Buist W, Veerkamp JH, et al. Associations of heart and adipocyte fatty acid-binding protein gene expression with intramuscular fat content in pigs. J Anim Sci. 2001;79(2):347–54.

28. Yagi H, Chen G, Yokoyama M, Hirata K, Augustus A, Kako Y, et al. Lipoprotein lipase (LPL) on the surface of cardiomyocytes increases lipid uptake and produces a cardiomyopathy. J Clin Invest. 2003;111(3):419–26.

29. Walsak R, Tontonoz P, PPARDiments and PPARDoxes: expanding roles for PPARD in the control of lipid metabolism. J Lipid Res. 2002;43(2):177–86.

30. Rosen ED, Walkey CJ, Pulsipher P, Spiegelman BM. Transcriptional regulation of adipogenesis. Genes Dev. 2001;15(11):1293–307.

31. Atkinson R, Ingham R, H{n}a{n} N, Liddle C, Atkins AR, Downes M, et al. PPAR {gammal} signaling and metabolism: the good, the bad and the future. Nat Med. 2013;19(5):557–66.

32. Albuquerque A, Neves JA, Redondeiro M, Laranjo M, Felix MR, Freitas A, et al. Long term betaine supplementation regulates genes involved in lipid and cholesterol metabolism of two muscles from an obese pig breed. Meat Sci. 2017;124:25–33.

33. Saha AK, Ruderman NB. Malonyl-CoA and AMP-activated protein kinase: an expanding partnership. Mol Cell Biochem. 2003;253(1–2):65–70.

34. Xue B, Kahn BB. AMPK integrates nutrient and hormonal signals to regulate food intake and energy balance through effects in the hypothalamus and peripheral tissues. J Physiol Lond. 2006;574(1):73–83.

35. Cai D, Wang J, Ji Y, Liu H, Yuan M, Dong H, et al. Gestational dietary betaine supplementation suppresses hepatic expression of lipogenic genes in neonatal piglets through epigenetic and glucocorticoid receptor-dependent mechanisms. Biochim Biophys Acta. 2016;1861(1):41–50.

36. Hardie DG, Ross FA, Hawley SA. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol. 2012;13(4):251–62.

37. Lee WJ, Kim M, Park HS, Kim HS, Jeon MJ, Oh KS, et al. AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARα and PGC-1. Biochem Biophys Res Commun. 2006;340(1):291–5.

38. Fein P. The biology of peroxisome proliferator-activated receptors. Diabetes. 2004;53(suppl 1):S43–50.

39. Matthews JO, Southern LL, Higbee AD, Persica MA, Bidner TD. Effects of betaine on growth, carcass characteristics, pork quality, and plasma metabolites of finishing pigs. J Anim Sci. 2001;79(3):722–8.

40. Martins JM, Neves JA, Freitas A, Trapicos J. Research article Betaine supplementation affects the cholesterol but not the lipid profile of pigs. Eur J Lipid Sci Technol. 2010;112:295–303.