Livestock Science 126 (2009) 130–135

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Betaine suppresses carnitine palmitoyltransferase I in skeletal muscle but not in liver of finishing pigs

Qi-Chun Huang [a,b], Xin-Yan Han [a,*], Zi-Rong Xu [a], Xiao-Yan Yang [b], Tong Chen [b], Xin-Tian Zheng [b]

[a] Key Laboratory of Molecular Animal Nutrition, Ministry of Education, Institute of Feed Science, Zhejiang University, No. 164 Qiutao North Road, Hangzhou 310029, People's Republic of China

[b] Key Laboratory of Preventive Veterinary Medicine and Biotechnology, Fujian Universities, College of Life Science, Longyan University, No. 1 Dongxaio North Road, Longyan 364000, People's Republic of China

ARTICLE INFO

Article history:
Received 1 December 2008
Received in revised form 28 May 2009
Accepted 12 June 2009

Keywords:
Betaine
Carnitine palmitoyltransferase I
Finishing pigs
mRNA

ABSTRACT

An experiment was conducted to investigate the effect of dietary betaine supplementation on the enzyme activity and mRNA abundance for carnitine palmitoyltransferase I (CPT I) in liver and skeletal muscle of finishing pigs. Forty-eight crossbred barrows and gilts [Duroc × (Seghers × Seghers)] weighing about 55 kg were divided into two dietary treatments, each with three replicates of eight pigs (four barrows and four gilts) per replicate. Pigs were fed a corn–soybean meal basal diet supplemented with betaine at 0 or 1250 mg/kg feed for 42 days. At trial termination, two pigs (one barrow and one gilt) weighing about 90 kg were selected from each replicate (six pigs per dietary treatment) and slaughtered for analyses. The results showed that intramuscular fat content in the *longissimus* muscle of pigs fed betaine was 23.6% higher than that of controls ($P<0.05$), whereas hepatic fat content was not affected with dietary betaine treatment. Muscle-type CPT I (M-CPT I) activity, but not liver-type CPT I (L-CPT I) activity was decreased by betaine supplementation. Furthermore, betaine supplementation reduced M-CPT I mRNA abundance by 14.6% ($P<0.05$) but did not affect L-CPT I mRNA abundance. There was a positive correlation between enzyme activity and mRNA abundance for both L-CPT I and M-CPT I ($r=0.67$ and $r=0.72$ for L-CPT I and M-CPT I, respectively; $P<0.05$). The study suggests that betaine may be involved in fat partitioning in pigs by reducing the activity and mRNA abundance of M-CPT I, with a resultant increase in intramuscular fat content.

1. Introduction

Betaine is a naturally occurring compound present in relatively large quantities in sugar beet and aquatic invertebrates but not present in significant quantities in most animal feedstuffs. Chemically, betaine is trimethylglycine, and the physiological function of betaine is either as an organic osmoprotectant or as a methyl donor which may partially reduce the requirements for other methyl donors (e.g., methionine, choline) and participate in lipid metabolism (Saunderson and Mackinlay, 1990; Kidd et al., 1997; Simon, 1999; Eklund et al., 2005).

The use of betaine in commercial swine diets has increased since Cadogan et al. (1993) reported a 14.8% decrease in backfat thickness of pigs fed betaine-supplemented diets. Up to now, most of the studies with betaine in pigs suggests that betaine supplementation may depress overall fat deposition, and therefore this study is focused on the potential stimulatory effects of betaine on lipid oxidation as a possible mechanism to explain fat reduction in finishing pigs. Fat deposition in adipose tissue represents a balance between fat synthesis and fat degradation (Chilliard, 1993). Feng (1996) and Huang et al. (2006, 2008) indicated that addition of betaine to the diet of finishing pigs resulted in decreased carcass fat deposition by either increasing the rate of lipolysis and (or) decreasing the rate of lipogenesis.

Carnitine palmitoyltransferase I (CPT I, *EC 2.3.1.21*), an integral outer mitochondrial membrane enzyme, catalyzes the

* Corresponding author. Institute of Feed Science, Zhejiang University (Huajiachi Campus), No. 164 Qiutao North Road, Hangzhou 310029, People's Republic of China. Tel.: +86 571 86091820; fax: +86 571 86994963.
E-mail address: xyhan@zju.edu.cn (X.-Y. Han).

1871-1413/$ – see front matter.
doi:10.1016/j.livsci.2009.06.015

initial and regulatory step in the β-oxidation of fatty acids (McGarry and Brown, 1997). However, the effect of betaine supplementation on CPT I activity has not been investigated. Therefore, the objective of the present study was to evaluate the influence of betaine supplementation on the enzyme activity and mRNA abundance for CPT I in liver (L-CPT I) and skeletal muscle (M-CPT I) of finishing pigs.

2. Materials and methods

2.1. Animals and treatments

This study was approved by the Zhejiang University Institutional Animal Care and Use Committee. Forty-eight crossbred barrows and gilts [Duroc × (Seghers × Seghers)] weighing 55.7 ± 0.48 kg were randomly allotted to two dietary treatments on the basis of body weight and ancestry, and gender was equalized across treatments. Three replications (i.e. pen) of eight pigs (four barrows and four gilts) per replicate were used for each treatment. Pigs were fed a corn–soybean meal basal diet (Table 1) with or without betaine supplementation. Supplementation was 1250 mg betaine/kg feed. The basal diets were formulated to meet or exceed the nutrient requirements of finishing pigs (NRC, 1998).

The feeding experiment lasted 42 days after a 7-day adaptation period. All pigs were housed in a curtain-sided pig barn with 3.0 × 4.5-m pens and concrete floor. Each pen was equipped with a dry/wet feeder. Feed (in meal form) and water were provided for *ad libitum* consumption throughout the experiment.

2.2. Sample collection

The diet was sampled for chemical analyses. At the end of the feeding trial, 12 pigs (six pigs from each dietary treatment with one barrow and one gilt per replicate) weighing about 90 kg were selected to collect tissue samples. Pigs were stunned by electrical shock and exsanguinated. At slaughter, liver and skeletal muscle samples were rapidly removed, snap frozen in liquid nitrogen and stored at −70 °C until subsequent analyses. Liver samples were taken from the right lobe of the liver, and skeletal muscle samples were taken from the *longissimus* muscle at the 10th-rib of the carcass.

Table 1
Composition of the basal diet, as-fed basis (g/kg).

Ingredient		Nutrient [a]	
Corn	625.0	Digestible energy (MJ/kg)	12.97
Soybean meal	190.0	Crude protein	149.1
Wheat bran	148.0	Ether extract	38.1
$CaHPO_4$	12.0	Crude fibre	29.2
Limestone	10.0	Ash	53.3
Salt	4.0	Calcium	7.6
Lysine	1.0	Phosphorus	6.1
Pre-mix [b]	10.0	Lysine	7.4
		Methionine	2.1
		Cystine	2.5
		Threonine	5.2
		Tryptophan	1.9

[a] All of the data were analysed value except for digestible energy and tryptophan that were calculated using swine NRC (1998) value.
[b] Pre-mix provided the following in mg/kg diet: Cu 25, Fe 150, Mn 50, Zn 118, I 0.8, Se 0.3, Co 1, retinol 1.95, cholecalciferol 0.045, alpha-tocopherol 30, phytylmenaquinone 1.4, thiamine 1.2, riboflavin 3.6, pyridoxine 1.4, vitamin B_{12} 0.01, biotin 0.05, D-pantothenic acid 7, folic acid 0.72, niacin 16, choline 500.

2.3. Chemical analyses

Chemical analyses of the basal diets were carried out according to Association of Official Analytical Chemists (AOAC, 1995): method no. 954.01 for crude protein, no. 920.39 for crude fat, no. 978.10 for crude fibre, no. 927.02 for calcium, no. 965.17 for phosphorus, no. 982.30 for amino acids.

All liver and muscle samples were freeze-dried and then ground through a sieve (mesh size 2 mm). Crude protein and fat content were determined according to the AOAC (1995) methods and reported on dry matter basis. All analyses were done in duplicate.

2.4. Measurement of CPT I activity

Liver mitochondria were isolated by differential centrifugation as described by Mersmann et al. (1972). Muscle mitochondria were prepared by the method of Watmough et al. (1988), as modified by Power and Newsholme (1997). Liver mitochondria isolated from 1 g tissue was suspended in 1 ml of isolation medium, while muscle mitochondria isolated from 3 g tissue was suspended in 1 ml of isolation medium. Carnitine palmitoyltransferase I activity was analysed using the method of Bieber et al. (1972). The assay was based on measurement of the initial CoA-SH formation by the 5,5′-dithio-bis-(2-nitrobenzoic acid) (DTNB) reaction from palmitoyl-CoA by mitochondria samples with L-carnitine. Briefly, 50 µl buffer solution (containing 116 mmol/L Tris, 2.5 mmol/L EDTA, 2 mmol/L DTNB, 0.2% Triton X-100, pH 8.0) and 50 µl mitochondria suspension (0.5–1 mg mitochondrial protein per 50 µl) was added to four semi-microcuvettes (Greiner, Germany). After 5-min preincubation at 30 °C, 50 µl palmitoyl-CoA (1 mmol/L dissolved in double distilled water) was added to three cuvettes. The fourth cuvette was used as a blank, adding 50 µl water instead of palmitoyl-CoA. The reaction was then started by adding 5 µl L-carnitine solution (1.2 mmol/L dissolved in 1 mol/L Tris, pH 8.0), immediately followed by photometric measurement at 412 nm at 30 °C (Ultrospec 2000, Pharmacia Biotech Ltd, Uppsala, Sweden) for 180 s (Bremer and Norum, 1967). Activity was defined as nmol CoA-SH released/(min mg mitochondrial protein). The protein content of the mitochondria suspension was determined according to the method of Bradford (1976).

2.5. Determination of CPT I mRNA abundance

Total RNA was extracted from frozen liver and muscle using Trizol reagent as described by the manufacturer (Invitrogen Life Technologies, Gaithersburg, MD, USA), respectively. RNA concentration and purity were determined by measuring the absorbance at 260 and 280 nm. In addition, the integrity of RNA was confirmed by ethidium bromide staining of ribosomal RNA following gel electrophoresis.

Reverse transcription (RT) was performed in a 25 µl reaction volume containing 1 µg total RNA, 1.0 µg random hexamer (Promega, Madison, WI, USA), 5 µl of 5× reaction buffer, 2 µl of