animal

Impact of dietary betaine and conjugated linoleic acid on insulin sensitivity, protein and fat metabolism of obese pigs

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To determine possible mechanisms of action that might explain the nutrient partitioning effect of betaine and conjugated linoleic acid (CLA) in Iberian pigs and to address potential adverse effects, twenty gilts were restrictively fed from 20 to 50 kg BW Control, 0.5% betaine, 1% CLA or 0.5% betaine + 1% CLA diets. Serum hormones and metabolites profile were determined at 30 kg BW and an oral glucose test was performed before slaughter. Pigs were slaughtered at 50 kg BW and livers were obtained for chemical and histological analysis. Decreased serum urea in pigs fed betaine and betaine + CLA diets (11%; P = 0.0001) indicated a more efficient N utilization. The increase in serum triacylglycerol (58% and 28%, respectively; P = 0.0098) indicated that CLA and betaine + CLA could have reduced adipose tissue triacylglycerol synthesis from preformed fatty acids. Serum glucose, low-density lipoprotein (LDL) cholesterol and non-esterified fatty acids were unaffected. CLA and betaine + CLA altered serum lipids profile, although liver of pigs fed CLA diet presented no histopathological changes and triglyceride content was not different from Control pigs. Compared with controls, serum growth hormone decreased (20% to 23%; P = 0.0209) for all treatments. Although serum insulin increased in CLA, and especially in betaine + CLA pigs (28% and 83%; P = 0.0001), indices of insulin resistance were unaffected. In conclusion, CLA, and especially betaine + CLA, induced changes in biochemical parameters and hormones that may partially explain a nutrient partitioning effect in young pigs. Nevertheless, they exhibited weak, although detrimental, effects on blood lipids. Moreover, although livers were chemically and histologically normal, pigs fed CLA diet challenged with a glucose load had higher serum glucose than controls.

Keywords: betaine, CLA, insulin sensitivity, liver histology, pig

Implications

Addition of conjugated linoleic acid (CLA), and especially betaine + CLA, to the diet of young pigs induced changes in biochemical parameters and hormones that may partially explain the nutrient partitioning effect, and increased lean deposition previously observed, with favourable consequences for the consumers because of the production of pork meat with low fat content. In contrast, although livers of young pigs fed betaine + CLA diet were chemically and histologically normal, a weak detrimental effect on blood lipids was observed. Furthermore, pigs fed CLA diet had higher serum glucose than controls when challenged with a glucose load.

Introduction

Betaine and conjugated linoleic acid (CLA) have the potential to alter growth and body composition in swine. Betaine is an amino acid derivative (trimethylglycine or glycine betaine) present in most organisms and is distributed widely in nature. It is an obligatory intermediate in the catabolism of choline that accumulates in tissues under water or salt stress (Petronini *et al.*, 1992), and it has potential lipotropic effects (Barak *et al.*, 1993). In addition, as a methyl donor, via S-adenosyl-methionine, betaine may partially reduce the requirements for methionine and participate in protein and lipid metabolism (Kidd *et al.*, 1997). Most studies with betaine in pigs focus on the effect of betaine on growth performance, carcass characteristics and pork quality. Several reports have indicated that betaine could decrease carcass fat deposition and increase carcass lean in pigs (Matthews *et al.*, 2001; Fernández-Fígares *et al.*, 2002),

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Table 1 *Ingredients and chemical composition of the experimental diets (as-fed basis)*

	Diet			
Item	Control	Betaine	CLA	Betaine + CLA
Ingredients (%)				
Barley	90	90	90	90
Soyabean meal	2	2	2	2
NaCl	0.5	0.5	0.5	0.5
Vitamin/trace mineral premix[a]	0.3	0.3	0.3	0.3
Calcium carbonate	1.1	1.1	1.1	1.1
Dibasic calcium phosphate	1.2	1.2	1.2	1.2
L-Lys	0.51	0.51	0.51	0.51
L-Thr	0.1	0.1	0.1	0.1
L-His	0.015	0.015	0.015	0.015
Corn starch	3.265	2.765	3.265	2.765
Sunflower oil	1	1	–	–
BHT	0.0125	0.0125	0.0125	0.0125
CLA	–	–	1	1
Betaine	–	0.5	–	0.5
Chemical analysis (%)				
CP[b]	11.6	11.7	11.6	11.5
Lys[c]	0.81	0.81	0.81	0.81
Total lipids[c]	2.83	2.83	2.83	2.83
Calcium[c]	0.77	0.77	0.77	0.77
Phosphorus[c]	0.55	0.55	0.55	0.55
GE (kJ/g DM)[b]	18.51	18.47	18.41	18.50
ME (kJ/g DM)[b]	15.11	14.77	14.69	14.41

CLA = conjugated linoleic acid; BHT = butylated hydroxytoluene; GE = gross energy; ME = metabolizable energy; DM = dry matter.
[a]Provided per kg of complete diet: retinol, 3.38 mg as retinyl acetate; cholecalciferol, 56.3 μg; DL-α-tocopherol, 25.2 mg as DL-α-tocopheryl acetate; menadione, 1.5 mg as menadione sodium bisulfite; thiamine, 0.15 mg; riboflavin, 3 mg; pyridoxine, 0.15 mg; cyanocobalamin, 15 μg; folic acid, 15 μg; nicotinic acid, 22.5 mg; D-pantothenic acid, 15 mg as calcium pantothenate; Mn, 15 mg as $MnSO_4.4H_2O$; Fe, 75 mg as $FeSO_4.7H_2O$; Zn, 120 mg as ZnO; I, 450 μg as KI; Cu, 60 mg as $CuSO_4.5H_2O$; Co, 300 μg as $CoSO_4.7H_2O$.
[b]Determined.
[c]Estimated values (FEDNA, 2003).

especially under restricted energy intake (Suster *et al.*, 2004) conditions. In contrast, CLA is a collective name that refers to a mixture of positional and geometric conjugated isomers of linoleic acid. Interest in feeding CLA to pigs has increased in the last decade as a result of its potential to increase rate of gain (Thiel-Cooper *et al.*, 2001), improve feed efficiency, protein accretion rate (Ostrowska *et al.*, 1999) and reduce body fat (Dugan *et al.*, 1997; Thiel-Cooper *et al.*, 2001), although the results have not been consistent. Most of the studies investigating the effects of betaine or CLA have been made with finishing lean pigs; interestingly, a most pronounced effect of CLA on back fat depth was found in fatter pigs (Dunshea *et al.*, 2005). In a recent study, a synergistic effect of dietary betaine and CLA on the growth of young growing Iberian pigs and carcass composition was found (Fernández-Fígares *et al.*, 2008), with betaine or CLA alone showing intermediate values. Nevertheless, little research has been conducted to investigate the pathways through which they might mediate their effect on growth and carcass traits. Furthermore, there is little information on how betaine and CLA affect serum hormone and metabolite profile. In addition, some deleterious effects have been reported when CLA was fed to laboratory animals, such as insulin resistance, liver enlargement and hepatic steatosis (Larsen *et al.*,

2003). Thus, the effect of dietary CLA on liver function needs to be researched in non-rodent models. In this paper, the effect of dietary CLA on liver fat content and morphology of young obese pigs was studied. In addition, the possibility of CLA-induced insulin resistance was addressed. Furthermore, we have evaluated changes in metabolic hormones and biochemical profile of young growing Iberian pigs fed betaine, CLA- or betaine + CLA-supplemented diets, with the final aim of providing information about their possible mechanisms of action in nutrient partitioning.

Material and methods

Animals and dietary treatments

A total of 20 purebred Iberian (Silvela strain) gilts from the same farrowing group were used in the study. Before the beginning of the trial, all pigs were group-housed and given *ad libitum* access to the Control diet between 16 and 20 kg BW. Further, they were housed in individual 2 m² pens located in a controlled-environment room (20 ± 1.5°C) and were randomly assigned to each dietary treatment. Diets were barley and soybean meal based and formulated to contain 12.0% crude protein (CP), 0.81% lysine and 14.8 MJ of metabolizable energy per kg of dry matter (DM) (Table 1).

The Control diet was not supplemented with betaine or CLA. Betaine diet was supplemented with 0.5% betaine (Betafin S1, crystalline, 96% purity; Danisco, Copenhagen, Denmark) at the expense of cornstarch. CLA diet was supplemented with 1% CLA (60% CLA isomers, half *cis*-9, *trans*-11 and half *trans*-10, *cis*-12 in methyl ester form; BASF, Ludwigshafen, Germany) at the expense of sunflower oil. CLA plus betaine diet was supplemented with 1% CLA and 0.5% betaine at the expense of sunflower oil and cornstarch, respectively. CP concentration was fixed to match the low potential for protein deposition of the growing Iberian pig, as determined previously by our group (Nieto *et al.*, 2002), and the remaining nutrients met or exceeded (National Research Council (NRC), 1998) requirements. Lysine, threonine and histidine were included in the crystalline form. Pigs were fed at 95% of *ad libitum* intake, calculated as a function of BW following the linear regression model described by Nieto *et al.* (2001) for Iberian pigs, and feed was distributed in two equal meals at 0900 and 1400 h. Daily allowance was calculated on the basis of the pig's BW, measured once per week. Refusals were collected on a daily basis and quantified, and pigs had free access to water throughout the experiment. Chemical analyses of the basal diet were carried out according to the methods of Association of Official Analytical Chemists (AOAC, 1990). The experimental protocol was reviewed and approved by the Bioethics Committee of the Spanish National Research Council (CSIC), and piglets were cared following the Spanish Ministry of Agriculture's guidelines (Boletín Oficial del Estado, 2005).

Experimental procedure

At 30 kg BW, pigs were fitted with jugular catheters. Pigs were fasted overnight before surgery and had free access to water. General anaesthesia was induced using an i.m. combination of Ketamine (15 mg/kg BW; Imalgene 1000, Merial, Barcelona, Spain)/Azaperone (2 mg/kg BW; Stresnil, Esteve Laboratories, Barcelona, Spain) and maintained throughout the preparation and surgical procedure by administering halothane (2%) and oxygen. Strict aseptic and sterile conditions were maintained along the whole procedure. An incision was made along the jugular furrow and the jugular vein was located and exposed. Catheter was inserted towards the auricular direction and sutured and secured to the vein. The catheter was exteriorized through the neck wall down and caudal from the ear. After patency was confirmed, the catheter was filled with physiological saline containing 250 IU heparin/ml and locked. Pigs were moved to metabolic cages and they resumed their normal eating habits the day after surgery. After recovery, on day 11, pigs were bled frequently (0, 2, 4, 6, 8, 12, 16, 20 h) and the blood samples were allowed to clot in ice for 3 h, centrifuged at $1000 \times$ **g** for 15 min and the serum samples were stored in different aliquots at $-80°C$ until analysis of serum metabolites and hormones.

An oral glucose tolerance test was performed on pigs fed Control and CLA diets the day before slaughter. Glucose tolerance test consisted of measuring serum glucose concentration after ingestion of 1 g glucose/kg BW in pigs fasting overnight at 0, 5, 10, 20, 30, 40, 60 120 and 180 min. At 50 kg BW, pigs were slaughtered, and immediately after slaughter, a sample of the liver was snap-frozen in liquid nitrogen and stored at $-80°C$ until chemical and histological analysis.

Analyses

All samples were assayed in duplicate except hormones that were assayed in triplicate. Chemical analyses of the basal diet were carried out according to the methods of AOAC (1990), as described by Fernández-Fígares *et al.* (2008). Hormones were measured using commercially available radio-immuno assay (RIA) or immuno-radiometric assay (IRMA) kits following the directions of the manufacturer, and the assays were validated for use in porcine serum in our laboratory (Fernández-Fígares *et al.*, 2007). Radioactivity in samples was measured using a gamma counter (Behring 1612; Nuclear Enterprises Ltd, Edinburgh, Scotland).

Growth hormone was analysed using a Linco porcine/canine RIA kit (catalogue no. PGH-46HK; Linco, St. Louis, MO, USA). The intra- and inter-assay coefficients of variation were 11.7% and 15.5%, respectively.

Leptin was analysed using a two-site IRMA (catalogue no. DSL-82100; Diagnostic Systems Laboratories, Webster, TX, USA). The intra- and inter-assay average coefficients of variation of concentrations were 3.7% and 4.0%, respectively.

Insulin was analysed using a Linco porcine insulin RIA kit (catalogue no. PI-12K; Linco, St. Louis, MO, USA). Human insulin was used as standard. The intra- and inter-assay coefficients of variation were 7.5% and 11.3%, respectively.

Insulin sensitivity was calculated using two indices:

- Test 1: Quantitative insulin sensitivity check index (QUICKI; Katz *et al.*, 2000) using the following formula:

$$QUICKI = 1/[\log(10) \text{ Fasting serum insulin, } \mu U/ml + \log(10) \text{ Fasting serum glucose, mg/dl}]$$

- Test 2: Homeostasis model assessment (HOMA; Matthews *et al.*, 1985) using the following formula:

$$HOMA = (\text{Fasting serum insulin, } \mu U/ml) \times (\text{Fasting serum glucose, mM})/22.5$$

IGF-1 was analysed using a two-site IRMA (catalogue no. DSL-5600; Diagnostic Systems Laboratories). The analytical procedure included an extraction step in which IGF-1 was separated from its binding proteins in serum for the detection of total IGF-1. The assay used antibodies against human IGF-1, and used human IGF-1 as the standard. The intra- and inter-assay coefficients of variation were 2.3% and 3.3%, respectively. Intra-assay coefficients of variation were calculated by analysing three serum pools of low, medium and high concentration of each hormone, assayed in triplicate. The same serum pools assayed for intra-assay coefficient of variation were analysed in two different assays to estimate inter-assay coefficient of variation.

Serum creatinine, urea, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides (TG) were determined colorimetrically

using an automated Advia 1650 apparatus (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA).

Serum non-esterified fatty acid (NEFA) concentration was assayed using the enzymatic spectrophotometric NEFA C ACS–ACOD (acyl-CoA synthetase–acyl-CoA oxidase) method (Wako Chemicals GmbH, Neuss, Germany).

Serum glucose was determined using Precision PCx Blood Glucose Test Strips (Abbott Laboratories, MediSense Products; Bedford, MA, USA).

Responses of serum glucose to the oral glucose challenge test was evaluated by computing areas under the response curves (AUC), determined using trapezoidal geometry for the 3-h period following glucose administration.

For histological analysis, the livers were thawed and cross-sections of liver from each pig were excised and fixed in 10% buffered formalin fixative. After routine processing, livers were embedded in paraffin, sectioned at 5 μm and stained with haematoxylin–eosin and reticulin stain for optical examination. Micrographs of liver were taken at a final magnification of 40× or 200× using an optical microscope (Olympus BX40-F3; Olympus Optical Co., Ltd, Tokyo, Japan) and a camera (Olympus SC30, Olympus Optical Co., Ltd). In the histological study, we assessed the presence of steatosis, cytoplasmic vacuolization (glycogen or other causes) and architectural changes: fibrosis (increase in conjunctive tissue), inflammation (portal and/or lobular), necrotic or neoplastic changes and deposits (iron, bilirubin, glycogen).

Liver lipid content was determined by chloroform–methanol 2 : 1 extraction of the freeze-dried sample using a Soxhlet apparatus (Selecta, Barcelona, Spain).

Statistical analyses

Data were evaluated using a mixed ANOVA with repeated measures (PROC MIXED, SAS Institute Inc., Cary, NC, USA), with treatment, time of sampling and their interaction in the model statement. Concentration at time zero of the analyte was included as a covariate in the statistical analysis.

For glucose tolerance test, area under the curve corrected for baseline (AUC, 0 to 180 min or fractions) was analysed using the GLM procedure of SAS. Significant differences among treatments were assessed using the Duncan multiple-range test (Duncan, 1955).

Least square means and pooled standard error of the means (s.e.m.) are presented. Differences were considered significant at $P < 0.05$ and trends were considered for $P < 0.10$.

Results

Growth and carcass composition parameters have been reported elsewhere (Fernández-Fígares et al., 2008). In brief, pigs fed betaine + CLA diets had increased average daily gain and carcass protein deposition, and carcass fat content tended to decrease compared with Control pigs, with intermediate values for pigs fed betaine and CLA diets, indicating synergistic action.

Biochemical serum variables

Biochemical serum parameters are shown in Table 2. Pigs fed betaine-supplemented diets had significantly ($P < 0.05$) lower serum urea, creatinine and HDL cholesterol/total cholesterol (11.1%, 7.0% and 10.9%, respectively) compared with Control pigs. Addition of CLA to the diet decreased ($P < 0.05$) serum creatinine and HDL cholesterol/total cholesterol (4.2% and 11.1%, respectively), tended ($P = 0.090$) to decrease serum urea (4.5%), increased ($P < 0.05$) total cholesterol and TG (11.1% and 58.1%, respectively) and tended to increase ($P = 0.052$) HDL cholesterol (4.3%) compared with Control pigs. Finally, compared with Control pigs, animals fed diets supplemented with both metabolic modifiers had reduced ($P < 0.05$) urea, creatinine, HDL cholesterol and HDL cholesterol/total cholesterol (10.7%, 6.5%, 9.0% and 9.6%, respectively), increased total cholesterol ($P < 0.05$; 8.0%), LDL/HDL

Table 2 Biochemical serum variables and liver fat of growing Iberian pigs fed diets supplemented or not with CLA (1%), betaine (0.5%) or betaine + CLA between 20 and 30 kg BW (repeated measures over 20 h with feed offered at 0 and 5 h relative to sampling)[1]

Item	Diet				s.e.m.	P-value
	Control	Betaine	CLA	Betaine + CLA		
Glucose (mmol/l)	6.0[a]	5.8[a]	6.0[a]	6.1[a]	0.13	NS
Urea (mmol/l)	8.0[a]	7.1[b]	7.6[a]	7.1[b]	0.15	0.0001
Creatinine (μmol/l)	63.5[a]	59.1[b]	60.8[b]	59.3[b]	0.73	0.0001
Total cholesterol (mmol/l)	2.40[a]	2.44[ac]	2.67[b]	2.59[bc]	0.060	0.017
HDL cholesterol (mmol/l)	0.97[ab]	0.95[a]	1.01[b]	0.88[c]	0.015	0.0001
LDL cholesterol (mmol/l)	1.53[a]	1.55[a]	1.61[a]	1.60[a]	0.032	NS
HDL/total cholesterol	0.44[a]	0.39[b]	0.39[b]	0.32[c]	0.013	0.0001
LDL/HDL cholesterol	1.64[a]	1.67[a]	1.64[a]	1.83[b]	0.040	0.043
TG (mmol/l)	0.38[a]	0.43[a]	0.61[b]	0.49[a]	0.034	0.0098
NEFA (mg/l)	42[a]	32[a]	36[a]	46[a]	6.1	NS
Liver fat (g/kg)	150[a]	147[a]	146[a]	158[a]	5	NS

CLA = conjugated linoleic acid; HDL = high-density lipoprotein; LDL = low-density lipoprotein; TG = triglycerides; NEFA = non-esterified fatty acid.
[a,b,c]Means within a row without a common superscript are different ($P < 0.05$).
[1]$n = 5$ pigs/group. Least square means of 8 samples (0, 2, 4, 6, 8, 12, 16, 20 h after the morning meal), except NEFA (0, 2 and 4 h). Treatment × Time interactions were not significant for all variables.

Table 3 *Serum hormones of growing Iberian pigs fed diets supplemented or not with CLA (1%), betaine (0.5%) or betaine + CLA from 20 to 30 Kg BW (repeated measures over 20 h with feed offered at 0 and 5 h relative to sampling)[1]*

	Diet					
Item	Control	Betaine	CLA	Betaine + CLA	s.e.m.	P-value
Growth hormone (μg/l)	3.0[a]	2.4[b]	2.3[b]	2.4[b]	0.17	0.021
IGF-1 (μg/l)	543[a]	519[a]	516[a]	564[a]	16	NS
Insulin (pmol/l)	278[a]	296[ab]	357[b]	509[c]	26	0.0001
Leptin (μg/l)	20.0[a]	20.3[a]	23.0[a]	22.2[a]	1.0	NS
Insulin sensitivity test 1[2]	0.284[a]	0.289[a]	0.288[a]	0.297[a]	0.007	NS
Insulin sensitivity test 2[3]	8.7[a]	7.9[a]	8.3[a]	6.3[a]	1.6	NS

CLA = conjugated linoleic acid.
[a,b,c]Means within a row without a common superscript are different ($P < 0.05$).
[1]n = 5 pigs/group. Least square means of 8 samples (0, 2, 4, 6, 8, 12, 16, 20 h after the morning meal). Treatment × Time interactions were not significant for all variables.
[2]QUICKI = 1/[log(10) Fasting serum insulin, μU/ml + log(10) Fasting serum glucose, mg/dl].
[3]HOMA = (Fasting serum insulin, μU/ml) × (Fasting serum glucose, mM)/22.5.

cholesterol ($P < 0.05$; 11.6%) and a trend to increased TG ($P = 0.054$; 27.9%).

Serum glucose, LDL cholesterol and NEFA were not affected by any dietary treatment ($P > 0.10$). Treatment × Time interactions were not significant for all variables.

Serum hormones profile
Metabolic hormone profile is shown in Table 3. Treatment × Time interactions were not significant for all variables.

Pig serum IGF-1 and leptin were not affected ($P > 0.10$) by dietary supplementation with betaine, CLA or both additives.

Betaine supplementation decreased serum growth hormone concentration ($P < 0.05$; 20%) compared with Control pigs. When diets were supplemented with CLA, a decrease ($P < 0.05$; 23%) in growth hormone concentration and an increase in insulin concentration ($P < 0.05$; 28.8%) were found compared with Control pigs. Similarly, when both betaine and CLA were added to the diet, growth hormone levels decreased and insulin levels increased sharply compared with Control pigs (20% and 83%, respectively).

Insulin sensitivity assessed by insulin sensitivity tests 1 and 2 was not different between treatments.

Oral glucose tolerance test
AUC were numerically higher for CLA pigs at all time intervals but only attained statistical significance at the 0 to 120 min interval (70%, $P < 0.05$; Table 4).

Liver histology and fat content
All livers showed lack of pathological changes, using light microscopy assessment. Representative histological sections of Control and CLA livers, respectively, are shown in Figures 1 and 2. At 40× magnification (Figures 1a and 2a), there was lack of widened conjunctive walls or fibrosis (arrows). At 200× magnification (Figures 1b and 2b), hepatocytes without vacuolization (no noticeable steatosis), inflammatory cells (lymphocytes or neutrophils), deposits or other pathological changes were observed. Nevertheless, we saw a widespread freezing artefact in samples of all treatments.

Table 4 *Area under the glucose curve (mmol/l and d) of 49 kg BW Iberian pigs fed Control or CLA diets and challenged with an oral glucose tolerance test (1 g/kg BW)[1]*

	Diet			
AUC	Control	CLA	s.e.m.	P-value
0–180 min	2684[a]	3726[a]	651	0.321
0–120 min	1693[a]	2884[b]	335	0.049
0–60 min	1119[a]	659[a]	204	0.121
0–40 min	887[a]	1089[a]	179	0.475
0–30 min	618[a]	807[a]	124	0.344
0–20 min	384[a]	556[a]	95	0.267
0–10 min	162[a]	222[a]	53	0.472
0–5 min	92[a]	120[a]	29	0.535

CLA = conjugated linoleic acid.
[a,b]Means within a row without a common superscript are different ($P < 0.05$).
[1]n = 5 pigs/group.

Furthermore, the histopathological findings were supported by the lack of differences in fat content among treatments (147 to 158 mg/g DM; $P > 0.10$; Table 2).

Discussion

This study was designed to investigate possible mechanisms involved in the increased protein deposition and decreased fat content previously shown in the carcass of pigs fed diets supplemented with betaine and CLA (Ostrowska *et al.*, 1999 and 2003; Fernández-Fígares *et al.*, 2002 and 2008; Mitchell *et al.*, 2005). Serum metabolites and hormones that may indicate a modification in protein or energy metabolism were investigated in this study. As some deleterious effects have been reported when CLA was fed to laboratory animals, such as insulin resistance and hepatic steatosis (Tsuboyama-Kasaoka *et al.*, 2000; Clément *et al.*, 2002), a glucose tolerance test was performed and liver morphology was studied to address these issues in a non-rodent model.
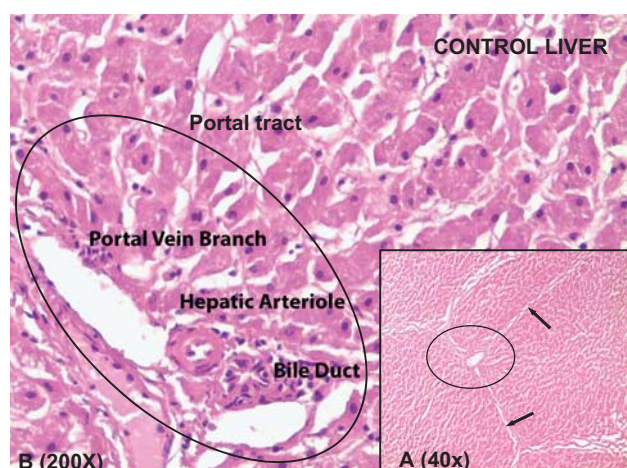
Figure 1 Microphotograph of a histological section of liver of pigs fed the Control diet. The arrows show lack of widened conjunctive walls or fibrosis. The ovals mark a portal tract containing the bile duct, the hepatic arteriole and the portal vein branch.
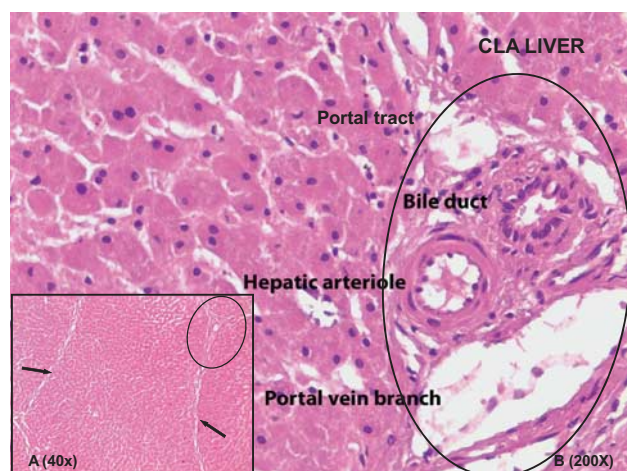

Figure 2 Microphotograph of a histological section of the liver of pigs fed the conjugated linoleic acid (CLA) diet. The arrows show lack of widened conjunctive walls or fibrosis. The ovals mark a portal tract containing the bile duct, the hepatic arteriole and the portal vein branch.

Effects on lipid metabolism
Possible mechanisms of decreased fat accretion include the inhibition of fat deposition from both preformed fatty acids (esterification) or glucose (*de novo* lipogenesis) and/or an increase in fat mobilization (lipolysis) in pigs fed dietary betaine or CLA.

The level of serum glucose, which is the primary carbon source of *de novo* lipogenesis, was monitored in this study. Isotope kinetics studies have shown that over 40% of whole body glucose pool can be used by adipose tissue for *de novo* lipogenesis in 80 kg pigs (Dunshea *et al.*, 1992). The uptake of glucose is dependent on insulin, and thus the level of this hormone was also examined. Reduced rates of glucose utilization for lipid synthesis could be a mechanism by which betaine or CLA reduce body fat. In this study, betaine or CLA had little effect on serum glucose. Our results would imply

that the differences in fat deposition in pigs fed betaine + CLA (Fernández-Fígares *et al.*, 2008) could not be explained by dissimilar availability of glucose for lipogenesis. Similarly, CLA did not alter plasma glucose and insulin in growing (Ramsay *et al.*, 2001) and finishing pigs (Ostrowska *et al.*, 2002) or lactating sows (Bontempo *et al.*, 2004). Nevertheless, serum glucose concentration was decreased in finishing pigs (Huang *et al.*, 2006) fed betaine-supplemented diets, the effect being ascribed to increased insulin levels. In our study, however, we encountered increased serum insulin in pigs fed CLA and betaine + CLA, with no change in serum glucose. Insulin stimulates lipogenesis (Kersten, 2001) and inhibits lipolysis (Bremer and Osmundsen, 1984). Nevertheless, a trend to increased serum TG in the betaine + CLA pigs in this experiment together with a trend to decreased carcass fat content in betaine + CLA pigs (Fernández-Fígares *et al.*, 2008) would suggest that insulin sensitivity could be partly impaired at adipose tissue level, and part of the glucose destined for lipid synthesis could be redirected to other tissues. Interestingly, glycaemia was not affected, which suggests that glucose uptake was encouraged in other tissues (liver and muscle). Nevertheless, *in vitro* studies have shown that CLA decreased gluconeogenesis in primary culture of porcine hepatocytes (Conde-Aguilera *et al.*, 2011) with no change in glycogen synthesis and degradation.

Higher levels of circulating triacylglycerol in pigs fed CLA were found in this experiment. Likewise, others have also found increased plasma triacylglycerol in pigs fed CLA (Stangl *et al.*, 1999; Ostrowska *et al.*, 2002) or betaine (Martins *et al.*, 2010). An increase in serum triacylglycerol levels could be an indication of reduced activity of lipoprotein lipase, which is in line with decreased heparin-releasable lipoprotein lipase activity by CLA in 3T3-L1-cultured murine adipocytes (Park *et al.*, 1997). The increase in serum TG levels observed when pigs were fed CLA and to a lesser extent betaine + CLA indicated that fat accretion could be reduced via decreased lipid synthesis from preformed fatty acids, which is in line with decreased carcass fat content of these pigs reported previously (Fernández-Fígares *et al.*, 2008). Nevertheless, Tischendorf *et al.* (2002) found no effect of CLA on plasma TG levels of finishing pigs. A reduced uptake of preformed fatty acids is indicated by the already-mentioned higher levels of circulating triacylglycerol in pigs fed diets containing CLA. Work with 3T3-L1 adipose cells supported the evidence that CLA enhanced lipolysis (Park *et al.*, 1997). However, contrary to Ostrowska *et al.* (2002), we have found no serum NEFA change in pigs fed CLA diets and similar results were found in lactating sows (Bontempo *et al.*, 2004). In this study, betaine had no effect in serum NEFA and similar results have previously been reported (Matthews *et al.*, 1998; Øverland *et al.*, 1999). Furthermore, it is interesting to point out that whole body or hepatic fatty acid oxidation was not affected by betaine in growing pigs (Wray-Cahen *et al.*, 2004), suggesting that reduction in adipose accretion must be via a mechanism other than oxidation. Nevertheless, Huang *et al.* (2006) reported increased NEFA but no change in TG of finishing pigs fed diets supplemented with betaine.

Inconsistency may be in part because of different dietary nutrient composition.

Effects on protein metabolism
In the somatotrophic axis, growth hormones can act in tissues directly on the cells itself or indirectly through the action of locally and systemically produced IGF-1 (Brook *et al.*, 1988; Florini *et al.*, 1996). In our experiment, pigs fed betaine and (or) CLA had reduced serum growth hormone, with no change in IGF-1 levels, which agree with the findings of Bontempo *et al.* (2004) in lactating sows fed CLA. By contrast, Li *et al.* (1999) found that CLA increases serum IGF-1 in rats, suggesting the hypothesis that CLA modulates body mass through mechanisms involving the IGF system. Nevertheless, decreased growth hormone levels in our experiment would contribute to an increase in lipogenic enzyme activities, as well as augmented adipocyte insulin sensitivity (Louveau and Gondret, 2004). This is in contrast with increased protein deposition and decreased carcass fat content in Iberian pigs fed betaine + CLA diets (Fernández-Fígares *et al.*, 2008), and would indicate that the mechanism by which these metabolic modifiers altered nutrient partitioning is not via the somatotrophic axis in Iberian pigs.

Although insulin is the primary hormone responsible for maintenance of glucose homeostasis, it also has potent actions on protein metabolism. The increased protein anabolism in response to feeding in previously fasted animals has been postulated to be mediated by the transient increases in insulin and amino acid concentrations that follow a meal (Garlick and Grant, 1988). We have found increased serum insulin in pigs fed CLA and betaine + CLA diets compared with Control pigs. Hyperinsulinaemia in these pigs raises the question of a possible insulin resistance induced by CLA alone or in the presence of betaine, which will be addressed later.

Coma *et al.* (1995) reported that N retention and urea N concentration were reflective of each other such that urea N concentration is minimized as N retention is maximized. Our data indicated that betaine and betaine + CLA supplementation did significantly decrease serum urea concentration compared with Control pigs, suggesting dissimilar protein metabolism. Similar results were also reported in finishing pigs (Matthews *et al.*, 1998; Huang *et al.*, 2007) fed betaine. On the contrary, Martins *et al.* (2010) in Alentejano finishing pigs reported no effect of betaine on plasma urea. Matthews *et al.* (1998) suggested that the effects of betaine on protein status of pigs were dependent on the CP and energy content of the diet. Regarding CLA, supplementation of the diet for 8 days did not alter plasma urea of finishing pigs (Ostrowska *et al.*, 2002), which is consistent with our results. Decreased serum urea in our research may suggest that betaine and betaine + CLA may improve N utilization in growing Iberian pigs fed diets supplemented with betaine or betaine + CLA. Although the responses to insulin on protein metabolism sharply decrease with age (Davis *et al.*, 2010), it could be speculated that increased serum insulin could stimulate muscle protein synthesis and/or inhibit muscle

proteolysis (Tesseraud *et al.*, 2007) in pigs fed betaine + CLA diets, which would be in line with decreased serum urea and the increased carcass protein deposition, as previously reported (Fernández-Fígares *et al.*, 2008).

Adverse side effects
Insulinaemia, glycaemia, liver steatosis and morphology. Hepatic steatosis is closely associated with decreased sensitivity of the liver to insulin, which may lead to hyperglycaemia and hyperinsulinaemia.

The impact of dietary CLA on hepatic steatosis and insulin sensitivity is controversial because of differences in species, isomer responses and degree and sensitivity to changes in adiposity (Tsuboyama-Kasaoka *et al.*, 2003; Nagao *et al.*, 2005; Wang *et al.*, 2006). In this study, we investigated the effects of dietary CLA and betaine in a highly adipogenic pig model.

It is usually considered that hyperinsulinaemia is reflective of insulin resistance. In our study, however, no differences in serum glucose were encountered among treatments, in spite of increased insulin levels in pigs fed CLA and betaine + CLA diets, and we did not see a change in insulin sensitivity calculated using insulin sensitivity tests 1 and 2. As the insulin sensitivity indices used are based on fasting glucose and insulin levels, it could be speculated that decreased insulin sensitivity in pigs fed CLA and betaine + CLA diets is postprandial. Moreover, liver fat was not affected by treatments in spite of increased serum insulin in pigs fed CLA and especially betaine + CLA. Interestingly, when pigs fed CLA diets were challenged with an oral glucose tolerance test, an increase in serum glucose was found compared with Control pigs, 2 h after the load.

No change in liver fat content or liver morphology assessed through optical microscopy was observed for any treatment. To our knowledge, this is the first report on the effects of CLA or betaine in liver morphology of pigs. In line with the lack of effect of CLA on liver fat content, TG content in primary culture of porcine hepatocytes increased in parallel with concentration of free fatty acids in the media, but CLA had no effect compared with linoleic acid (Conde-Aguilera *et al.*, 2011). In rats, there are conflicting results. Although rats fed 2% CLA diets for 2 weeks showed lipid accumulation in the liver as observed through electron microscopy (Yamasaki *et al.*, 2000a), dietary CLA fed at 1% prevented steatosis observed histologically in livers of rats fed high fructose diets (Kostogrys and Pisulewski, 2010).

Although there is some evidence from mice and human studies that the CLA isomer *trans*-10, *cis*-12 may produce liver hypertrophy and insulin resistance via a redistribution of fat deposition that resembles lipodystrophy (Larsen *et al.*, 2003), the CLA-induced changes in glucose tolerance and liver metabolism observed in the various animal studies are conflicting. For example, dietary CLA has been reported to decrease TG accumulation in the liver of Wistar rats (Purushotham *et al.*, 2007), but hyperinsulinaemia was observed in wild-type mice supplemented with CLA (Tsuboyama-Kasaoka *et al.*, 2000).

In relation to betaine, there is a scarcity of information regarding its effect on liver lipids in non-rodent models.

It has been reported (Barak *et al.*, 1993) that adding betaine as a methyl donor stimulates liver lipid mobilization in alcoholic fatty liver of rats, but we have been unable to show differences in liver fat content of pigs fed betaine diet compared with Control pigs, may be because lipogenesis in pig liver is negligible and also because of the fact that the growing pig does not mobilize fat from adipose tissue and would not be expected to accumulate fat in the liver.

The adipocyte-derived hormone leptin has a profound impact on steatosis and insulin sensitivity, and there seems to be a crosstalk between the leptin and the insulin signalling pathways at the levels of the insulin receptor substrate and phosphatidylinositol-3 kinase (Ceddia, 2005).

Leptin has been implicated in feed intake regulation, reproductive and immune function (Barb *et al.*, 1999). Because leptin is secreted mainly by adipocytes, circulating levels correlate directly with body mass index in humans (Ahima and Flier, 2000), with a positive correlation between circulating leptin levels and adiposity also reported in pigs (Barb *et al.*, 2001). As CLA is reported to reduce body fat content (Ostrowska *et al.*, 2003), a reduction in plasma leptin levels would be expected in CLA-supplemented animals (Kang and Pariza, 2002) unless there is central or peripheral leptin resistance. Leptin, by binding to its receptors located in skeletal muscle and fat cells promotes energy dissipation and prevents fatty acid accumulation in these tissues (Ceddia, 2005). In contrast, under conditions of peripheral leptin resistance, the activation of pathways involved in fatty acid oxidation may be impaired, which would lead to intracellular accumulation of lipid intermediates and cause insulin resistance (Steinberg and Dyck, 2000). Serum leptin concentration in this experiment is much higher than in finishing Berkshire, Chester White, Duroc, Landrace, Poland China or Yorkshire pigs (Berg *et al.*, 2003). We showed that Iberian pigs had higher plasma leptin concentration than Landrace gilts at 20 kg BW (Fernández-Fígares *et al.*, 2007); however, feed intake was not diminished, which suggests that they are leptin resistant. In this study, although some evidence of CLA-induced insulin resistance was detected (hyperinsulinaemia, increased serum glucose after glucose tolerance test), insulin resistance is not yet established as similar glucose levels, liver fat levels, and insulin sensitivity tests were found in CLA and Control pigs.

We have found no information in the literature on the effects of betaine on circulating leptin concentration in any experimental model. The effects of dietary CLA on serum leptin have not been extensively investigated in pigs. In our experiment, there was no effect of any treatment on serum leptin concentration. Dietary CLA reduced serum leptin levels in rats, mice and humans (Delany *et al.*, 1999; Medina *et al.*, 2000; Yamasaki *et al.*, 2000b), whereas it was increased in lactating sows (Bontempo *et al.*, 2004).

Serum lipids and arteriosclerosis. Although increased total cholesterol (Matthews *et al.*, 2001; Martins *et al.*, 2010) and decreased HDL/total cholesterol (Martins *et al.*, 2010) have been reported in pigs fed betaine, other authors reported no

betaine effect on cholesterol, plasma NEFA or TG (Matthews *et al.*, 1998; Øverland *et al.*, 1999). We found decreased HDL/total cholesterol in betaine pigs, but no significant effects in total cholesterol or fractions. However, in humans, Turpin (1985) reported that adding betaine as a methyl donor reduced hyperlipidaemia.

Stangl *et al.* (1999) found increased LDL-to-HDL ratio and a trend to augmented total and HDL cholesterol in CLA-supplemented pigs compared with Control pigs receiving sunflower oil. Nevertheless, Tischendorf *et al.* (2002) found no differences in TG or cholesterol fractions and concluded that blood lipid status of CLA fed pigs did not represent arteriosclerosis risk. Pigs fed betaine + CLA diets had diminished HDL/total cholesterol, increased LDL/HDL cholesterol and serum TG, which may be considered a risk for coronary heart disease (Kinosian *et al.*, 1994).

In conclusion, our observations show that betaine and CLA had an impact on protein and fat metabolism such that different hormones and biochemical parameters were affected. Decreased serum urea would indicate improved efficiency of protein utilization, and increased serum TG would suggest that fat is being mobilized. The use of betaine + CLA may worsen, from a human health point of view, the ratio between cholesterol fractions, which together with increased serum TG, may indicate a risk of atherogenesis. In addition, although CLA had a moderate effect on insulin sensitivity, no negative effects were found in liver histology for any treatment.

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References

Ahima RS and Flier JS 2000. Leptin. Annual Reviews of Physiology 62, 413–437.

Association of Official Analytical Chemists (AOAC) 1990. Official methods of analysis, 15th edition. AOAC, Washington, DC.

Barak AJ, Beckenhauer HC, Junnila M and Tuma DJ 1993. Dietary betaine promotes generation of hepatic S-adenosylmethionine and protects the liver from ethanol-induced fatty infiltration. Alcoholism, Clinical and Experimental Research 17, 552–555.

Barb CR, Hausmann GJ and Houseknecht KL 2001. Biology of leptin in the pig. Domestic Animal Endocrinology 21, 297–317.

Barb CR, Barrett JB, Kraeling RR and Rampacek GB 1999. Role of leptin in modulating neuroendocrine function: a metabolic link between the brain-pituitary and adipose tissue. Reproduction in Domestic Animals 34, 111–125.

Berg EP, McFadin EL, Maddock KR, Goodwin RN, Baas TJ and Keisler DH 2003. Serum concentrations of leptin in six genetic lines of swine and relationship with growth and carcass characteristics. Journal of Animal Science 81, 167–171.

Boletín Oficial del Estado 2005. Real Decreto Español 1201/2005 sobre la protección de los animales utilizados para experimentación y otros fines científicos. Boletín Oficial del Estado 252, 34367–34391.

Bontempo V, Sciannimanico D, Pastorelli G, Rossi R, Rosi F and Corino C 2004. Dietary conjugated linoleic acid positively affects immunological variables in lactating sows and piglets. Journal of Nutrition 134, 817–824.

Bremer J and Osmundsen H 1984. Fatty acid oxidation and its regulation. In Fatty acid metabolism and its regulation (ed. S Numa), pp. 113–153. Elsevier Science Publishers, Amsterdam, The Netherlands.

Brook CG, Hindmarsh PC and Stanhope R 1988. Growth and growth hormone secretion. Journal of Endocrinology 119, 179–184.

Ceddia RB 2005. Direct metabolic regulation in skeletal muscle and fat tissue by leptin: implications for glucose and fatty acids homeostasis. International Journal of Obesity 29, 1175–1183.

Clément L, Poirier H, Niot I, Bocher V, Guerre-Millo M, Krief S, Staels B and Besnard P 2002. Dietary trans-10,cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. Journal of Lipid Research 43, 1400–1409.

Coma J, Carrion D and Zimmerman DR 1995. Use of plasma urea nitrogen as a rapid response criterion to determine the lysine requirement of pigs. Journal of Animal Science 73, 472–481.

Conde-Aguilera JA, Lachica M, Nieto R and Fernández-Fígares I 2011. Metabolic regulation of fatty acid esterification and effects of CLA on glucose homeostasis in pig hepatocytes. Animal, In press. doi:10.1017/S1751731111001613.

Davis TA, Suryawan A, Orellana RA, Fiorotto ML and Burrin DG 2010. Amino acids and insulin are regulators of muscle protein synthesis in neonatal pigs. Animal 4, 1790–1796.

Delany JP, Blohm F, Truett AA, Scimeca JA and West DB 1999. Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake. American Journal of Physiology 276, 1172–1179.

Dugan MER, Aalhus JL, Schaefer AL and Kramer JKG 1997. The effect of conjugated linoleic acid on fat to lean repartitioning and feed conversion in pigs. Canadian Journal of Animal Science 77, 723–725.

Duncan DB 1955. Multiple range and multiple F-test. Biometrics 11, 1–42.

Dunshea FR, Harris DM, Bauman DE, Boyd RD and Bell AW 1992. Effect of porcine somatotropin on in vivo glucose kinetics and lipogenesis in the growing pig. Journal of Animal Science 70, 141–151.

Dunshea FR, D'Souza DN, Pethick DW, Harper GS and Warner RD 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Science 71, 8–38.

Fernández-Fígares I, Lachica M, Nieto R, Rivera-Ferre M and Aguilera JF 2007. Serum profile of metabolites and hormones in obese (Iberian) and lean (Landrace) growing gilts fed balanced or lysine deficient diets. Livestock Science 110, 73–81.

Fernández-Fígares I, Conde-Aguilera JA, Nieto R, Lachica M and Aguilera JF 2008. Synergistic effects of betaine and conjugated linoleic acid on growth and carcass composition of growing Iberian pigs. Journal of Animal Science 86, 102–111.

Fernández-Fígares I, Wray-Cahen D, Steele NC, Campbell RG, Hall DD, Virtanen E and Caperna TJ 2002. Effect of dietary betaine on energy utilization and partitioning in the young growing feed restricted pig. Journal of Animal Science 80, 421–428.

Florini JR, Ewton DZ and Coolican SA 1996. Growth hormone and the insulin-like growth factor system in myogenesis. Endocrine Reviews 17, 481–517.

Garlick PJ and Grant I 1988. Amino acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin. Effect of branched-chain amino acids. Biochemical Journal 254, 579–584.

Huang QC, Xu ZR, Han XY and Lia WF 2006. Changes in hormones, growth factor and lipid metabolism in finishing pigs fed betaine. Livestock Science 105, 78–85.

Huang QC, Xu ZR, Han XY and Li WF 2007. Effect of betaine on growth hormone pulsatile secretion and serum metabolites in finishing pigs. Journal of Animal Physiology and Animal Nutrition 91, 85–90.

Kang K and Pariza MW 2002. Trans-10, cis-12 conjugated linoleic acid reduces leptin secretion from 3T3-L1 adipocytes. Biochemical and Biophysical Research Communications 287, 377–382.

Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G and Quon MJ 2000. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. Journal of Clinical Endocrinology and Metabolism 85, 2402–2410.

Kersten S 2001. Mechanisms of nutritional and hormonal regulation of lipogenesis. EMBO Reports 2, 282–286.

Kidd MT, Ferket PR and Garlich JD 1997. Nutritional and osmoregulatory functions of betaine. World's Poultry Science Journal 53, 125–139.

Kinosian B, Glick H and Garland G 1994. Cholesterol and coronary heart disease: predicting risks by levels and ratios. Annals of Internal Medicine 121, 641–647.

Kostogrys RB and Pisulewski PM 2010. Effect of conjugated linoleic acid (CLA) on lipid profile and liver histology in laboratory rats fed high-fructose diet. Environmental Toxicology and Pharmacology 30, 245–250.

Larsen TM, Toubro S and Astrup A 2003. Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and human studies. Journal of Lipid Research 44, 2234–2241.

Li Y, Seifert MF, Ney DM, Grahn M, Grant AL, Allen KG and Watkins BA 1999. Dietary conjugated linoleic acids alter serum IGF-I and IGF binding protein concentrations and reduce bone formation in rats fed (n-6) or (n-3) fatty acids. Journal of Bone Mineral Research 14, 1153–1162.

Louveau I and Gondret F 2004. Regulation of development and metabolism of adipose tissue by growth hormone and the insulin-like growth factor system. Domestic Animal Endocrinology 27, 241–255.

Martins JM, Neves JA, Freitas A and Tirapicos JL 2010. Betaine supplementation affects the cholesterol but not the lipid profile of pigs. European Journal of Lipid Science and Technology 112, 295–303.

Matthews JO, Southern LL, Pontif JE, Higbie AD and Bidner TD 1998. Interactive effects of betaine, crude protein, and net energy in finishing pigs. Journal of Animal Science 76, 2444–2455.

Matthews JO, Southern LL, Higbie AD, Persica MA and Bidner TD 2001. Effects of betaine on growth, carcass characteristics, pork quality, and plasma metabolites of finishing pigs. Journal of Animal Science 79, 722–728.

Matthews DR, Hosker JP, Rudenki AS, Naylor BA, Treacher DF and Turner RC 1985. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28, 412–419.

Medina EA, Horn WF, Keim NL, Havel PJ, Benito P, Kelley DS, Nelson GJ and Erickson KL 2000. Conjugated linoleic acid supplementation in humans: effects on circulating leptin concentrations and appetite. Lipids 35, 783–788.

Mitchell AD, Pursel VG, Elsasser TH, Mcmurtry JP and Bee G 2005. Effects of dietary conjugated linoleic acid on growth and body composition of control and IGF-I transgenic pigs. Animal Research 54, 395–411.

Nagao K, Inoue N, Wang YM, Shirouchi B and Yanagita T 2005. Dietary conjugated linoleic acid alleviates nonalcoholic fatty liver disease in Zucker (fa/fa) rats. Journal of Nutrition 135, 9–13.

Nieto R, Miranda A, García MA and Aguilera JF 2002. The effect of dietary protein content and feeding level on the rate of protein deposition and energy utilization in growing Iberian pigs from 15 to 50 kg body weight. British Journal of Nutrition 88, 39–49.

Nieto R, Lara L, García MA, Gómez F, Zalvide M, Cruz M, Pariente JM, Moreno A and Aguilera JF 2001. Evaluation of an integrated feeding system in the Iberian pig. Study of food consumption and productive parameters. Solo Cerdo Iberico 6, 57–69.

National Research Council (NRC) 1998. Nutrient requirements of swine, 10th edition. National Academy Press, Washington, DC.

Ostrowska E, Muralitharan M, Cross RF, Baumann DE and Dunshea FR 1999. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. Journal of Nutrition 129, 2037–2042.

Ostrowska E, Cross RF, Muralitharan M, Bauman DE and Dunshea FR 2002. Effects of dietary fat and conjugated linoleic acid on plasma metabolite concentrations and metabolic responses to homeostatic signals in pigs. British Journal of Nutrition 88, 625–634.

Ostrowska E, Suster D, Muralitharan M, Cross RF, Leury BJ, Bauman DE and Dunshea FR 2003. Conjugated linoleic acid decreases fat accretion in pigs: evaluation by dual-energy X-ray absorptiometry. British Journal of Nutrition 89, 219–229.

Øverland M, Rørvik KA and Skrede A 1999. Effect of trimethylamine oxide and betaine in swine diets on growth performance, carcass characteristics, nutrient digestibility, and sensory quality of pork. Journal of Animal Science 77, 2143–2153.

Park Y, Albright KJ, Liu W, Storkson JM, Cook ME and Pariza MW 1997. Effect of conjugated linoleic acid on body composition in mice. Lipids 32, 853–858.

Petronini PG, de Angelis EM, Borghetti P, Borghetti AF and Wheelers KP 1992. Modulation by betaine of cellular responses to osmotic stress. Biochemical Journal 282, 69–73.

Purushotham A, Shrode GE, Wendel AA, Liu LF and Belury MA 2007. Conjugated linoleic acid does not reduce body fat but decreases hepatic steatosis in adult Wistar rats. Journal of Nutritional Biochemistry 18, 676–684.

Ramsay TG, Evock-Clover CM, Steele NC and Azain MJ 2001. Dietary conjugated linoleic acid alters fatty acid composition of pig skeletal muscle and fat. Journal of Animal Science 79, 2152–2161.

Stangl GI, Muller H and Kirchgessner M 1999. Conjugated linoleum acid effects on circulating hormones, metabolites and lipoproteins, and its proportion in fasting serum and erythrocyte membranes of swine. European Journal of Nutrition 38, 271–277.

Steinberg GR and Dyck DJ 2000. Development of leptin resistance in rat soleus muscle in response to high-fat diets. American Journal of Physiology Endocrinology and Metabolism 279, 1374–1382.

Suster D, Leury BJ, King RH, Mottram M and Dunshea FR 2004. Interrelationships between porcine somatotropin (pST), betaine and energy level on body composition and tissue distribution of finisher boars. Australian Journal of Agricultural Research 55, 983–990.

Tesseraud S, Métayer S, Duchêne S, Bigot K, Grizard J and Dupont J 2007. Regulation of protein metabolism by insulin: value of different approaches and animal models. Domestic Animal Endocrinology 33, 123–142.

Thiel-Cooper RL, Parrish JRFC, Sparks JC, Wiegand BR and Ewan RC 2001. Conjugated linoleic acid changes swine performance and carcass composition. Journal of Animal Science 79, 1821–1828.

Tischendorf F, Möckel P, Schöne F, Plonné M and Jahreis G 2002. Effect of dietary conjugated linoleic acids on the distribution of fatty acids in serum lipoprotein fractions and different tissues of growing pigs. Journal of Animal Physiology and Animal Nutrition 86, 313–325.

Tsuboyama-Kasaoka N, Miyazaki H, Kasaoka S and Ezaki O 2003. Increasing the amount of fat in a conjugated linoleic acid-supplemented diet reduces lipodystrophy in mice. Journal of Nutrition 133, 1793–1799.

Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, Kasai M, Ikemoto S and Ezaki O 2000. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes 49, 1534–1542.

Turpin G 1985. A double blind study of the effectiveness of Beaufor betaine citrate ampoules in the treatment of type IV hyperlipidaemias. Semaine des Hopitaux 61, 2429–2433.

Wang YM, Nagao K, Inoue N, Ujino Y, Shimada Y, Nagao T, Iwata T, Kamegai T, Yamauchi-Sato Y and Yanagita T 2006. Isomer-specific anti-obese and hypolipidemic properties of conjugated linoleic acid in obese OLETF rats. Bioscience, Biotechnology and Biochemistry 70, 355–362.

Wray-Cahen D, Fernández-Fígares I, Virtanen E, Steele NC and Caperna TJ 2004. Betaine improves growth, but does not induce whole body or hepatic palmitate oxidation in swine (Sus scrofa domestica). Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 137, 131–140.

Yamasaki M, Mansho K, Mishima H, Kimura G, Sasaki M, Kasai M, Tachibana H and Yamada K 2000a. Effect of dietary conjugated linoleic acid on lipid peroxidation and histological change in rat liver tissues. Journal of Agricultural and Food Chemistry 48, 6367–6371.

Yamasaki M, Mansho K, Ogino Y, Kasai M, Tachibana H and Yamada K 2000b. Acute reduction of serum leptin level by dietary conjugated linoleic acid in Sprague-Dawley rats. Journal of Nutritional Biochemistry 11, 467–471.