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Conjugated linoleic acid decreases fat accretion in pigs: evaluation by dual-energy X-ray absorptiometry

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Thirty female Large White × Landrace pigs (average weight 57·2 (SD 1·9) kg) were allocated to one of six dietary treatments containing 0, 1·25, 2·5, 5·0, 7·5 or 10·0 g 55 % conjugated linoleic acids (CLA) isomers (CLA-55)/kg diet and fed for 8 weeks. Each pig was scanned at 0, 28 and 56 d and again at post slaughter using dual-energy X-ray absorptiometry (DXA) to determine the temporal pattern of body composition responses. Values determined by DXA were adjusted using regression equations generated from validation experiments between chemically and DXA-predicted values. Overall, there was a significant linear reduction in fat content with the increasing levels of CLA in the diet ($P=0·007$, $P=0·011$, $P=0·008$ at week 4, week 8 and for the carcass, respectively). The greatest improvement was recorded at the early stages of CLA supplementation and for the highest dose of CLA (week 4, −19·2 % compared with week 8, −13·7 %). In the first 4 weeks of feeding CLA, pigs receiving 10 g CLA-55/kg diet deposited 93 g less fat/d than pigs fed basal diets ($P=0·002$) compared with only 6 g less fat than control animals in the final 4 weeks. Lean content and lean deposition rate were maximised at 5 and 2·5 g CLA-55/kg diet for the first 4 weeks ($P=0·016$) and the final 4 weeks of treatment ($P = 0·17$), respectively. DXA estimates of bone mineral content and bone mineral density were not affected by CLA supplementation throughout the experiment. These data demonstrate that dietary CLA decreases body fat in a dose-dependent manner and that the response is greatest over the initial 4 weeks of treatment.

Conjugated linoleic acid: Dual-energy X-ray absorptiometry: Body composition: Pig

Conjugated linoleic acid (CLA) represents a mixture of positional and geometric isomers (18 : 2*n*-6) of linoleic acid with conjugated double bonds located at positions 7,9-, 8,10-, 9,11-, 10,12- or 11,13- on the C chain. CLA exerts many positive health effects in experimental models including anticarcinogenic and anti-tumorogenic as well as anti-atherogenic and antidiabetogenic properties (for reviews, see Whigham *et al.* 2000; Pariza *et al.* 2001). Another biological effect of CLA relates to fat accretion and nutrient partitioning. Dietary CLA supplementation reduces body fat mass in rodents (Chin *et al.* 1994; Park *et al.* 1995; DeLany *et al.* 1999) and pigs (Ostrowska *et al.* 1999). These findings were based on experiments involving slaughter and subsequent chemical analysis in which the ultimate rates of change in body composition were determined at the conclusion of CLA supplementation. However, little is known about the longitudinal effects of CLA supplementation on body composition and generation of such data requires killing large numbers of animals at various stages of development. Alternatively, body composition can be measured in live animals and carcasses using dual-energy X-ray absorptiometry (DXA) (Mitchell *et al.* 1998; Lukaski *et al.* 1999; Suster *et al.* 2000), which is non-invasive and allows longitudinal studies in the same animal. The DXA apparatus can be used to analyse the fat, lean and bone mineral content (BMC) of the whole animal and is also capable of measuring the regional distribution of these tissues (Mitchell *et al.*

Abbreviations: BMC, bone mineral content; CLA, conjugated linoleic acid; DXA, dual-energy X-ray absorptiometry; FCE, feed conversion efficiency; R_F, attenuation of pure fat; R_L, attenuation of bone-free lean tissue; R_{ST}, ratio of soft tissue attenuation.

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1996; Suster *et al.* 2001). The aim of the present study was to utilise DXA technology to validate the effectiveness of CLA supplementation in pigs and to generate more comprehensive data on the progressive change in body composition in growing and finisher pigs fed different levels of dietary CLA.

Materials and methods

Animals and handling

All procedures involving animals were approved by the Victorian Institute of Animal Science Animal Ethics Committee (Anonymous, 1997). Thirty-five (Large White × Landrace) pigs were used in the experiment. To obtain initial body composition by chemical analysis, five pigs were killed (after an *in vivo* DXA scan; see p. 220) at the beginning of the experiment. The remaining thirty pigs were randomly allocated to one of six dietary treatments (0, 1·25, 2·5, 5·0, 7·5 or 10·0 g CLA-55/kg diet (Natural Lipids Ltd., Hovdebygda, Norway). The CLA used in the present study contained 55 % (w/w) CLA isomers and was prepared from sunflower-seed oil. Therefore, the CLA concentrations for the six diets were 0, 0·7, 1·4, 2·75, 4·1 and 5·5 g/kg diet, respectively. The CLA content of the supplement and diets was measured by HPLC (Cross *et al.* 2000; Ostrowska *et al.* 2000). The CLA-55 mixture consisted of 13·8 % *trans*/*cis*-8,10, 24·5 % *cis*/*trans*-9,11, 30·5 % *trans*/*cis*-10,12 and 18·3 % *cis*/*trans*-11,3 isomers present in a non-esterified fatty acid form. The remainder of the CLA-55 mixture consisted of small amounts of *cis*, *cis* and *trans*, *trans* isomers.

Pigs were initially blocked into groups of six pigs with an average live weight of 57·2 (SD 1·9) kg and P2 back fat (65 mm from the midline over the last rib) thickness of 11·5 (SD 1·3) mm. The experimental diets (Table 1) were formulated to contain 14·3 MJ digestible energy, 9·3 g lysine and 175 g crude protein/kg air-dry diet, which was in excess of protein and lysine requirements for the class of pig used in the study (Dunshea *et al.* 1993). Amino acid content relative to lysine was kept in excess of the amino acid balance proposed as ideal by the Standing Committee on Agriculture (1987) to ensure that lysine was the first limiting amino acid. Diets were fed *ad libitum* for 8 weeks and unlimited access to water was provided via nipple drinkers. Pigs were individually housed to allow individual feed intake and live-weight measurements to be made weekly.

Dual-energy X-ray absorptiometry scans

Total whole body DXA scans were performed using a Hologic QDR 4500A Fan Beam X-Ray Bone Densitometer (Hologic, Inc., Waltham, MA) equipped with the body composition option software. The software calculates the ratio of soft tissue attenuation (R_{ST}) of the low- and high-energy beams as they pass through the body. The attenuations of pure fat (R_F) and of bone-free lean tissue (R_L) are known from theoretical calculation and *in vitro* measurements. Thus, an equation for each X-ray energy level with two unknown factors can be solved to calculate the proportion of fat (∞) and lean tissue (β) in each pixel containing soft tissue only (Hologic, Inc.):

$$R_{ST}\ (\text{low energy}) = \infty(R_F) + \beta(R_L),$$

$$R_{ST}\ (\text{high energy}) = \infty(R_F) + \beta(R_L).$$

On each scanning day, the unit was calibrated according to the manufacturer's specifications to ensure accurate fat and lean composition results, for accurate BMC measurement and to minimise baseline drift. Pigs were scanned at the beginning of the experiment, after 4 weeks and again at the conclusion of the experiment (after 8 weeks). Pigs were fasted for 16 h before scanning to minimise errors associated with water in the gut lumen. Fasted pigs were also weighed before scanning to assist with unit calibration.

Table 1. Composition of experimental diets (g/kg)*

	Dose of CLA-55 (g/kg diet)					
Ingredients	0	1·25	2·5	5·0	7·5	10·0
Wheat	691·74	691·74	691·74	691·74	691·74	691·74
Soyabean meal	121·61	121·61	121·61	121·61	121·61	121·61
Peas	100·00	100·00	100·00	100·00	100·00	100·00
Dicalcium phosphate	26·71	26·71	26·71	26·71	26·71	26·71
Blood meal	21·12	21·12	21·12	21·12	21·12	21·12
Limestone	10·90	10·90	10·90	10·90	10·90	10·90
L-lysine hydrochloride	2·00	2·00	2·00	2·00	2·00	2·00
NaCl	2·00	2·00	2·00	2·00	2·00	2·00
Vitamin and mineral premix†	2·00	2·00	2·00	2·00	2·00	2·00
DL-methionine	0·98	0·98	0·98	0·98	0·98	0·98
L-threonine	0·94	0·94	0·94	0·94	0·94	0·94
Soyabean oil	20·0	18·75	17·5	15·0	12·5	10·0
CLA-55	0·0	1·25	2·5	5·0	7·5	10·0

CLA-55, 55 % conjugated linoleic acid.

* The wheat-based diet was formulated to contain 14·3 MJ digestible energy and 175 g crude protein/kg air-dry diet.

† Provided the following nutrients (mg/kg air-dry diet: retinol, 6·4; cholecalciferol, 0·083; α-tocopherol, 22; menadione, 0·60; riboflavin, 3·3; nicotinic acid, 16·5; pantothenic acid, 5·5; pyrodoxine, 1·1; biotin, 0·56; choline, 1100; cyanocobalamin, 0·017; Fe, 88; Zn, 55; Mn, 22; Cu, 6·6; I, 0·22; Se, 0·1.

Pigs were anaesthetised using an intra-muscular injection of Xylazine (20 mg Xylazine as hydrochloride/ml; Troy Labs Pty. Ltd., Smithfield, NSW) and Ketamine (100 mg Ketamine as hydrochloride/ml; Troy Labs Pty. Ltd.) at 0·05 and 0·1 ml/kg body weight, respectively. This intra-muscular injection was followed by respiratory administration of Isoflourane (Abbott Aust. Pty. Ltd., Kurmel, NSW) to eliminate any further movement. For the final scan before slaughter, Thiopentone (10 % Thiopentone Sodium; Jorox Pty. Ltd., Silverwater, NSW) was administered intravenously at 0·7 ml/kg body weight in place of Ketamine and Xylazine. Sedated animals were placed prone on the scan table, on their stomach with front and back legs extended caudally. This orientation was used to accommodate the DXA software, which was designed on a basis of a laterally recumbent human subject, i.e. lying on his or her back. Small plastic air-filled bags, undetectable by DXA, were placed between the body and front legs to provide the clearance required by the software to perform accurate analysis.

Slaughter and carcass dual-energy X-ray absorptiometry scans

After CO_2 anaesthesia induced using a commercial CO_2 stunning unit, pigs were exsanguinated, had hair removed and were eviscerated. Internal organs were weighed and the gastrointestinal tract emptied and reweighed. Internal organs and empty gastrointestinal tract were then frozen at −20°C for analysis of fat, water and protein content. Carcasses were hung in the chiller from the hindlimbs, and had their front legs tied towards the dorsal end before the onset of rigor. This allowed the correct body positioning required for DXA scanning of carcasses, which was carried out 24 h post chilling. The carcasses were wrapped in plastic to minimise any moisture loss. Post scanning, the empty carcasses were hung overnight at 4°C before being split down the midline and frozen. After freezing, the right side of each carcass and the empty viscera were ground up separately and prepared for chemical analyses (Campbell *et al.* 1985). DM and tissue moisture was determined by drying samples to constant weight in a force-draught oven at 105°C. Ash was determined by combustion in a muffle furnace at 600°C for 5 h. Protein was determined by Kjeldahl analysis as modified by Helrich (1990) and fat was extracted from freeze-dried samples by chloroform-methanol (2:1, v/v) extraction (Folch *et al.* 1957). Lean tissue was determined from the summation of tissue protein and water. Both halves of the carcass were assumed to be equal in weight and chemical composition and the results were doubled for whole carcass estimation.

Calculation and statistical analysis

The DXA values obtained in the present study were corrected using regression equations obtained from a larger study involving 150 pigs ranging from 10 to 130 kg live weight and also scanned using the Hologic QDR4500 DXA (Suster *et al.* 2000).

The effects of different levels of CLA in the diet on body composition were analysed by ANOVA suitable for a dose response with linear and quadratic effects determined. The model included block, replicate and CLA dose. In addition, comparisons were made between diets with or without CLA. For these analyses the model included block and CLA dose. All analyses were performed using GENSTAT for Windows, version 4.1 (Payne *et al.* 1993).

Results

Dietary CLA had no significant effect on average daily gain throughout the present study (Table 2). However, in general there was a linear reduction ($P=0·04$) in feed intake with increasing dose of CLA. This reduction in feed intake was more pronounced over the first 4 weeks of treatment, although a trend towards a linear reduction in feed intake was still evident between weeks 4 and 8 ($P=0·09$). Consequently, feed conversion efficiency (FCE) was increased by dietary CLA, particularly over the first 4 weeks of the treatment. During the initial 4 weeks of the study there was a quadratic ($P=0·03$) increase in FCE with the effect maximised at 5 g CLA-55/kg diet. When all CLA treatments were pooled, including CLA in the diet increased FCE by 8·1 % ($P=0·006$) over the first 4 weeks while there was no significant change between weeks 4 and 8 ($P=0·26$).

While live weight at the end of the experiment was unaffected by CLA supplementation, carcass weight increased in a quadratic fashion with the highest carcass weights being observed in pigs fed 5 g CLA-55/kg diet (83·8 *v.* 86·6 kg for pigs fed diets supplemented with 0 and 5 g/kg, respectively; $P=0·006$) (Table 3). Dressing percentage was not affected by the CLA supplementation (Table 3).

Appropriate blocking and allocation to treatments ensured that there were no significant differences in DXA-derived estimates of fat, lean, BMC and bone density at the beginning of the experiment (data not shown). The whole-body fat content of pigs fed diets supplemented with CLA decreased in a linear fashion ($P=0·007$ and $P=0·01$ at weeks 4 and 8, respectively) with increasing level of dietary CLA (Table 4). The proportional reduction in fat content was greatest at week 4, where in general, CLA-fed pigs tended to have 10·3 % ($P=0·09$) less fat than the pigs fed the control diets (Table 4). At the highest level of supplementation (10 g CLA-55/kg) a 19 % reduction in body fat was observed (17·4 *v.* 14·1 kg; $P=0·007$). After 8 weeks of CLA supplementation, a smaller (−6 %) reduction in body fat was still evident in CLA-fed pigs but this was not significant ($P=0·32$). Again, the greatest improvement was observed at the highest level of supplementation with CLA-55 (−14 %). Significantly linear dose-dependent reductions in fat mass ($P=0·008$) were also observed in the carcass at the end of the study (Table 4).

Dietary CLA supplementation increased DXA-determined lean tissue composition in a quadratic manner throughout the entire experiment ($P=0·005$ and $P=0·01$ at weeks 4 and 8, respectively) (Table 4). After 4 weeks of treatment, the lean tissue response was maximised in pigs fed diets supplemented with 5 g CLA-55/kg (56·2 *v.* 58·6 kg). After 8 weeks of feeding, the effect was still maximised at a CLA-55 level around 2·5 to 5·0 g/kg

Table 2. Effect of dietary conjugated linoleic acid (CLA) on growth performance of finishing gilts*
(Mean values and standard errors of the difference)

	Rate of gain (g/d)			Feed intake (g/d)			Feed conversion efficiency		
Time (weeks)...	0–4	4–8	0–8	0–4	4–8	0–8	0–4	4–8	0–8
Dose of CLA-55 (g/kg)†									
0	1024	816	920	3083	3023	3053	0·33	0·28	0·30
1·25	1099	853	976	3062	3079	3070	0·36	0·28	0·32
2·5	1076	870	973	2895	2977	2936	0·37	0·29	0·33
5·0	1067	899	983	2811	2778	2795	0·38	0·32	0·35
7·5	987	851	919	2911	2932	2922	0·34	0·29	0·31
10·0	974	830	902	2677	2758	2717	0·36	0·30	0·33
CLA									
No	1024	816	920	3083	3023	3053	0·33	0·28	0·30
Yes	1041	861	951	2871	2905	2888	0·36	0·30	0·33
SED (dose)‡	84	83	69	197	188	174	0·012	0·025	0·016
SED (CLA)§	65	65	53	152	146	135	0·009	0·019	0·012
Significance (*P*)									
CLA (linear)	0·19	0·96	0·43	0·04	0·09	0·04	0·36	0·30	0·21
CLA (quadratic)	0·40	0·32	0·26	0·85	0·82	0·82	0·03	0·21	0·07
Control *v.* CLA	0·80	0·49	0·57	0·18	0·43	0·23	0·006	0·26	0·04

* For details of diets and procedures, see Table 1 and p. 220.
† CLA nominally contained 55 % fatty acids as CLA isomers.
‡ SED between different doses of CLA-55 (five per treatment group).
§ SED of the means between diets containing none (no, *n* 5) and some (yes, *n* 25) supplemental CLA-55.

(67·4 *v.* 70·7 kg). The lean tissue composition in pigs fed higher levels (>5 g/kg) of CLA-55 was not significantly different from the control pigs. A similar quadratic response ($P=0·009$) was also observed for DXA-predicted lean content of the carcass (Table 4). There was no significant effect of dietary CLA supplement on DXA-determined empty body and carcass ash content (Table 4).

As expected from the DXA-predicted body composition data, CLA supplementation caused a significant overall linear decrease ($P=0·008$) in the rate of fat deposition (Table 5). When pooled across dietary treatments, pigs fed CLA deposited 14·5 % less fat than control pigs over the initial period of 4 weeks of supplementation (311 *v.* 266 g/d for control pigs; $P=0·07$). The greatest reduction in fat deposition was observed in pigs fed the highest level of CLA (10 g CLA-55/kg diet) with a 30 % reduction in fat deposition relative to controls (311 *v.* 218 g/d for control pigs). The efficacy of CLA in reducing fat deposition diminished during the final 4 weeks of feeding, where pigs fed CLA were not different to the control pigs (287 *v.* 299 g/d for control pigs; $P=0·59$). However, the combination of the large reduction in fat deposition in the first 4 weeks of feeding, followed by little change over the final 4 weeks, still resulted in an overall significant ($P=0·008$) linear reduction in fat deposition in pigs with increasing levels of dietary CLA supplementation over the duration of the experiment.

Over the initial 4 weeks of feeding, the DXA-estimated lean deposition response to CLA supplementation was quadratic ($P=0·02$) in nature, being maximised at a dietary CLA inclusion rate of 5·0 g CLA-55/kg (Table 5). However, lean deposition rate during the final 4 weeks of treatment, pooled across CLA-supplemented pigs, was no different from control pigs (400 *v.* 420 g/d; $P=0·77$) with no evidence of a dose response. The magnitude of the response over the first 4 weeks was such that lean deposition rate increased in a quadratic manner ($P=0·017$) over the entire duration of the experiment, being maximised at a dietary CLA-55 inclusion of between 2·5 and 5 g/kg (Table 5).

The estimates of ash (Table 4) and bone mineral density (data not shown) in live animals and carcasses, as determined by DXA, were largely unaffected by CLA

Table 3. Effect of dietary conjugated linoleic acid (CLA) on live weight, carcass weight and dressing at the completion of the study*
(Mean values and standard effors of the difference)

	Live weight (kg)	Carcass (kg)	Dressing (g/kg)
Dose of CLA-55 (g/kg diet)†			
0	104·8	83·8	799
1·25	109·4	86·3	789
2·5	107·8	85·8	794
5·0	108·7	86·6	797
7·5	106·0	84·8	800
10·0	105·8	82·5	781
CLA			
No	104·8	83·8	799
Yes	107·6	85·2	792
SED (dose)‡	2·5	1·8	9·2
SED (CLA)§	1·9	1·4	7·2
Significance (*P*)			
CLA (linear)	0·60	0·32	0·27
CLA (quadratic)	0·17	0·006	0·21
Control *v.* CLA	0·17	0·47	0·36

* For details of diets and procedures see Table 1 and p. 220.
† CLA nominally contained 55 % fatty acids as CLA isomers.
‡ SED between different doses of CLA-55 (five per treatment group).
§ SED of the means between diets containing none (no, *n* 5) and some (yes, *n* 25) supplemental CLA-55.

Table 4. Adjusted dual-energy X-ray absorptiometry estimates of fat, lean and ash content of gilts fed dietary conjugated linoleic acid (CLA)*

(Mean values and standard errors of the difference)

	Fat mass (kg)			Lean mass (kg)			Ash mass (kg)		
Time (week)...	4	8	Carcass	4	8	Carcass	4	8	Carcass
Dose of CLA-55 (g/kg diet)†									
0	17·4	25·5	23·0	56·2	67·4	54·5	1·9	2·3	2·0
1·25	18·0	27·8	24·0	56·1	67·4	55·2	1·8	2·4	1·9
2·5	15·4	23·6	20·9	58·1	70·9	58·1	1·7	2·2	1·9
5·0	15·1	23·1	20·1	58·6	70·7	57·7	1·8	2·4	2·0
7·5	15·6	23·5	20·4	57·3	69·3	56·9	1·9	2·4	1·9
10·0	14·1	22·0	18·6	54·7	65·5	53·7	1·8	2·4	1·9
CLA									
No	17·4	25·5	23·0	56·2	67·4	54·5	1·9	2·3	2·0
Yes	15·6	24·0	20·8	57·0	68·7	56·3	1·8	2·4	1·9
SED (dose)‡	1·31	1·84	1·89	1·35	2·26	1·85	0·07	0·09	0·065
SED (CLA)§	1·02	1·43	1·47	1·04	1·75	1·44	0·05	0·07	0·050
Significance (*P*)									
CLA (linear)	0·007	0·01	0·008	0·41	0·50	0·69	0·78	0·21	0·74
CLA (quadratic)	0·55	0·67	0·67	0·005	0·01	0·009	0·69	0·61	0·62
Control *v.* CLA	0·09	0·32	0·14	0·46	0·45	0·23	0·53	0·85	0·13

* For details of diets and procedures, see Table 1 and p. 220.
† CLA nominally contained 55 % fatty acids as CLA isomers.
‡ SED between different doses of CLA-55 (five per treatment group).
§ SED of the means between diets containing none (no, *n* 5) and some (yes, *n* 25) supplemental CLA-55.

Table 5. Effect of dietary conjugated linoleic acid (CLA) determined by dual-energy X-ray absorptiometry on daily deposition rate of fat, lean and ash in gilts between weeks 0 and 4, 4 and 8 and the overall deposition rates (0–8 weeks)*

(Mean values and standard errors of the difference)

	Fat (g/d)			Lean (g/d)			Ash (g/d)		
Time (week)...	0–4	4–8	0–8	0–4	4–8	0–8	0–4	4–8	0–8
Dose of CLA-55 (g/kg diet)†									
0	311	287	299	585	400	493	20·2	17·4	18·8
1·25	319	350	334	631	401	516	20·6	19·7	20·1
2·5	277	294	285	655	456	556	18·1	15·8	17·0
5·0	251	285	268	656	434	545	21·5	20·6	21·0
7·5	267	283	275	606	426	516	21·3	18·5	19·9
10·0	218	281	249	550	385	467	19·7	21·0	20·4
CLA									
No	311	287	299	585	400	493	20·2	17·4	18·8
Yes	266	299	282	620	420	520	20·2	19·1	19·7
SED (dose)‡	30·79	27·43	25·22	43·40	49·00	35·30	1·753	1·984	1·531
SED (CLA)§	23·85	21·25	19·54	33·60	37·90	27·30	1·358	1·537	1·186
Significance (*P*)									
CLA (linear)	0·002	0·14	0·008	0·18	0·77	0·30	0·68	0·09	0·17
CLA (quadratic)	0·85	0·99	0·91	0·02	0·17	0·02	0·61	0·73	0·95
Control *v.* CLA	0·07	0·59	0·41	0·32	0·60	0·33	0·98	0·28	0·47

* For details of diets and procedures, see Table 1 and p. 220.
† CLA nominally contained 55 % fatty acids as CLA isomers.
‡ SED between different doses of CLA-55 (five per treatment group).
§ SED of the means between diets containing none (no, *n* 5) and some (yes, *n* 25) supplemental CLA-55.

supplementation. However, the deposition rate of ash tended to increase with increasing levels of dietary CLA between weeks 4 and 8 only (Table 5). There was no overall significant effect on bone deposition rate over the entire duration of the experiment ($P=0·17$).

Chemically determined carcass water composition increased linearly ($P=0·011$) with increasing dietary supplementation of CLA (Table 6). While there was a trend towards a linear increase ($P=0·09$) in carcass protein content with increasing CLA, overall difference between the chemical protein content of control pigs and that of the CLA pigs was not significant ($P=0·54$). Chemically determined carcass fat decreased linearly ($P=0·008$) with increasing dietary CLA content (Table 6). At the highest level of CLA inclusion, carcass fat content was decreased by approximately 43 g/kg (−15 %). Carcass ash content

Table 6. Effect of dietary conjugated linoleic acid (CLA) on chemical carcass composition at week 8 of the treatment*
(Mean values and standard errors of the difference)

	Protein (g/kg)	Water (g/kg)	Water:protein	Ash (g/kg)	Fat (g/kg)	Fat:protein
Dose of CLA-55 (g/kg diet)†						
0	159	517	3·26	26·2	293	1·85
1·25	151	516	3·44	25·2	303	2·05
2·5	164	547	3·33	23·7	261	1·61
5·0	168	570	3·39	25·9	232	1·39
7·5	163	545	3·35	25·8	262	1·61
10·0	165	554	3·37	27·1	250	1·52
CLA						
No	159	517	3·26	26·2	293	1·85
Yes	162	546	3·38	25·6	261	1·64
SED (dose)‡	5·9	16·2	0·131	1·36	19·8	0·183
SED (CLA)§	4·6	12·6	0·102	1·06	15·3	0·142
Significance (*P*)						
CLA (linear)	0·09	0·01	0·71	0·18	0·008	0·01
CLA (quadratic)	0·26	0·04	0·58	0·17	0·06	0·11
Control *v.* CLA	0·54	0·03	0·24	0·57	0·05	0·14

* For details of diets and procedures, see Table 1 and p. 220.
† CLA nominally contained 55 % fatty acids as CLA isomers.
‡ SED between different doses of CLA-55 (five per treatment group).
§ SED of the means between diets containing none (no, *n* 5) and some (yes, *n* 25) supplemental CLA-55.

was not affected by dietary CLA. Fat:protein in the carcass decreased linearly ($P=0·01$) with increasing dietary CLA. There were no significant differences in water:protein in the carcass.

As expected from the chemically predicted body composition data, the chemical fat deposition rates determined by the slaughter-balance technique showed a similar linear reduction ($P=0·01$) with the increasing level of dietary CLA in the feed (Table 7). At the highest dietary CLA content, there was an 84 g/d decrease in fat deposition (322 *v.* 238 g/d). While the effect of dietary CLA supplementation on chemical protein deposition was variable, there was a significant quadratic effect ($P = 0·02$), with the response being maximised at a dietary CLA-55 content of 5 g/kg (Table 7). However, there was no overall difference in protein deposition rate between control and all CLA-supplemented pigs (138 *v.* 141 g/d; $P=0·69$). Similarly, the water deposition rate determined by the slaughter-balance technique showed a quadratic response to the CLA level supplemented in the diet ($P = 0·003$; Table 7) with the response again maximised at 5·0 g CLA-55/kg diet. There was no effect of dietary CLA on chemical ash deposition.

The efficacy of using DXA to measure effects of CLA

Table 7. Effect of dietary conjugated linoleic acid (CLA) on tissue deposition rates determined by the slaughter-balance technique at week 8 of the treatment*
(Mean values and standard errors of the difference)

	Protein (g/d)	Water (g/d)	Lean (g/d)	Ash (g/d)	Fat (g/d)
Dose of CLA-55 (g/kg diet)†					
0	138	420	558	19·4	322
1·25	129	428	557	19·7	348
2·5	148	480	627	15·9	285
5·0	153	519	672	19·9	239
7·5	144	471	615	18·8	278
10·0	130	431	561	17·7	238
CLA					
No	138	420	558	19·4	322
Yes	141	466	607	18·4	277
SED (dose)‡	9·8	32·1	39·1	1·72	38·9
SED (CLA)§	7·6	24·9	30·3	1·33	30·1
Significance (*P*)					
CLA (linear)	0·94	0·47	0·56	0·59	0·01
CLA (quadratic)	0·02	0·003	0·003	0·97	0·41
Control *v.* CLA	0·69	0·08	0·12	0·46	0·15

* For details of diets and procedures, see Table 1 and p. 220.
† CLA nominally contained 55 % fatty acids as CLA isomers.
‡ SED between different doses of CLA-55 (five per treatment group).
§ SED of the means between diets containing none (no, *n* 5) and some (yes, *n* 25) supplemental CLA-55.

Table 8. Comparison of the final values (week 8) for dual-energy X-ray absorptiometry (DXA), DXA-adjusted values (DXA-adj) and chemical analysis (CHEM) of body composition*

(Mean values and standard deviations for thirty-five pigs)

	CHEM		DXA		DXAadj†		Significance (*P*)	
Variable	Mean	SD	Mean	SD	Mean	SD	CHEM *v.* DXA	CHEM *v.* DXAadj
Chemical analysis of carcass (including viscera) *v.* DXA live animal estimates								
Fat (kg)	25·55	3·94	19·42	2·94	24·23	3·87	<0·001	<0·001
Lean (kg)	68·24	4·19	80·57	4·76	68·52	3·95	<0·001	0·51
BMC (kg)	2·51	0·16	1·81	0·15	2·35	0·17	<0·001	<0·001
Chemical analysis of empty carcass *v.* DXA empty carcass estimates								
Fat (kg)	21·38	3·71	18·22	3·00	21·17	3·66	<0·001	0·51
Lean (kg)	56·20	3·66	59·89	3·53	56·02	3·35	0·002	0·63
BMC (kg)	2·05	0·15	1·72	0·10	1·94	0·11	<0·001	<0·001

BMC, bone mineral content.
* For details of procedures, see p. 220.
† DXA values were adjusted using equations from Suster *et al.* (2000).

on body composition were tested by comparing chemically determined body composition directly against the estimates of body composition generated by the internal algorithms (Table 8). An additional comparison was then made after the outputs from the DXA machine were corrected using regression equations relating chemical composition to DXA estimates generated in a larger (*n* 150) group of pigs of varying live weight and body composition (Suster *et al.* 2000). The whole animal and carcass DXA-predicted values for fat, lean and ash compared with those measured on the same animals directly by chemical analysis were all significantly different ($P<0·001$) with fat and ash being underestimated and lean being overestimated. The greatest differences were in the live animal estimations of lean, which was probably due to presence of water in the gut lumen detected as being associated with lean tissue. Hence, DXA estimation of lean in the empty body measurement was much closer to the chemically determined values, although they were still significantly different ($P=0·002$). The DXA predictions adjusted using the regression equations from DXA validation studies (Suster *et al.* 2000) essentially corrected the overestimations and underestimations made by DXA algorithms. Although whole-body fat and both whole-body and carcass ash were still significantly different after correction, the numerical differences were quite small. The adjusted DXA estimates of carcass fat, lean and BMC done on the live animal averaged 94·8, 100·4 and 93·6 % of the chemically determined values, respectively, while similar comparisons of DXA estimates determined on the empty carcass were 99·0, 99·7 and 94·6, respectively (Table 8).

The correlation (*R* 0·86) between the fat deposition rate estimated by the slaughter-balance technique *v.* the DXA estimations of fat deposition illustrates that the two methods are measuring the same thing (Fig. 1). The slope of the relationship is also close to unity (1·07), although the intercept is significantly different than zero indicating a 20 g bias towards the chemical prediction.

Dietary CLA supplementation did not appear to have any significant effect on the weights of any of the major internal organs and tissues (Table 9).

Discussion

While there were no significant changes in growth rate as a result of CLA supplementation, feed intake decreased in a linear fashion ($P=0·04$) as the concentration of CLA in the diet increased. Consequently, there was a significant improvement in FCE for CLA-fed pigs (+7·9 %; $P=0·045$). The significant effects in FCE were mostly due to improvements during the first 4 weeks of the treatment interval where FCE was increased by 8 % ($P=0·006$). Other workers have also observed similar small improvements in FCE in pigs supplemented with dietary CLA (Dugan *et al.* 1997; Thiel-Cooper *et al.* 2001) and our earlier work demonstrated that FCE was increased by 6·4 % by CLA supplementation (Ostrowska *et al.* 1999).

The present study confirms the findings from our earlier reports showing that dietary CLA reduces fat deposition while increasing lean deposition in finisher pigs (Dunshea *et al.* 1998; Ostrowska *et al.* 1999). Using DXA to measure composition has shown that the response was most pronounced during the first 4 weeks of CLA supplementation with very little response thereafter. For example, fat deposition during the first 4 weeks was minimised at the

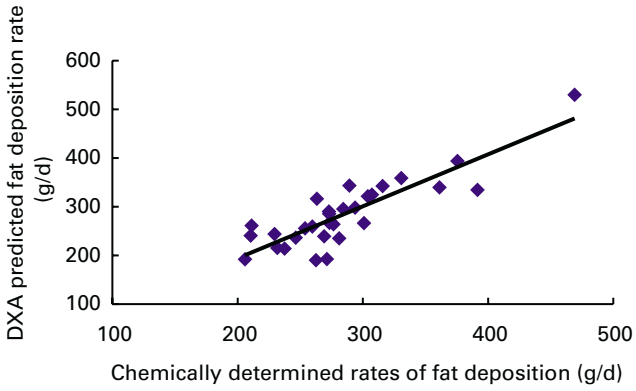

Fig. 1. Correlation between body fat deposition rates determined by slaughter-balance techniques dual-energy X-ray absorptiometry (DXA)-predicted fat deposition rates ($Y = 1·07X - 19·6$, R 0·86, $P<0·001$).

Table 9. Effect of dietary conjugated linoleic acid (CLA) on visceral organ weights at week 8 of the treatment*
(Mean values and standard errors of the difference)

	Weight (g)								
Organ...	Liver	Heart	Kidneys	Lungs	Stomach	Small intestine	Caecum	Large intestine	Spleen
Dose of CLA-55 (g/kg diet)†									
0	1757	333	339	630	601	1377	220	1851	154
1·25	1743	337	302	535	570	1282	199	1558	134
2·5	1814	346	326	554	597	1316	226	1693	160
5·0	1782	380	360	536	553	1279	221	1828	143
7·5	1880	366	338	669	561	1230	196	1600	130
10·0	1718	353	353	577	542	1214	207	1669	146
CLA									
No	1757	333	339	630	601	1377	220	1851	154
Yes	1787	356	336	574	565	1264	210	1670	142
SED (dose)‡	111	20·7	24·2	85·6	41·7	104	24·3	157	15·6
SED (CLA)§	86	16·0	18·7	66·3	32·3	80	18·8	121	12·1
Significance (*P*)									
CLA (linear)	0·86	0·12	0·13	0·70	0·15	0·12	0·53	0·55	0·45
CLA (quadratic)	0·29	0·09	0·93	0·64	0·86	0·81	0·82	0·91	0·62
Control *v.* CLA	0·73	0·17	0·89	0·41	0·27	0·18	0·59	0·15	0·37

* For details of diets and procedures, see Table 1 and p. 220.
† CLA nominally contained 55 % fatty acids as CLA isomers.
‡ SED between different doses of CLA-55 (five per treatment group).
§ SED of the means between diets containing none (no, *n* 5) and some (yes, *n* 25) supplemental CLA-55.

highest level of CLA supplementation, with pigs receiving 10 g CLA-55/kg diet depositing 93 g less fat/d than pigs fed the unsupplemented diets. However, over the final 4 weeks, fat deposition in the same group of pigs was only 6 g/d less than that of control pigs. Despite this, the combination of large differences in fat deposition rates over the first 4 weeks of feeding with much smaller differences during the following weeks of CLA supplementation still resulted in an overall significant decrease in fat deposition. At the end of the experiment, pigs fed the highest level of CLA averaged 50 g/d less fat accretion than control pigs ($P=0{\cdot}008$). In a previous experiment, the reduction in fat deposition rate at the highest level of supplementation (10 g/kg) was found to be much greater (−86 g/d; Ostrowska *et al.* 1999). However, in the latter study the fat deposition rates were determined from the difference in body composition found in pig carcasses at the end of the experiment compared with a representative group of pigs slaughtered at the beginning of the experiment (slaughter-balance technique). The present study also provided a very similar estimate of reduction in fat deposition when estimated using the slaughter-balance technique (−84 g/d). However, there is a very high internal consistency in fat deposition responses both between studies using the same technique and within a study using different techniques (Fig. 1).

The DXA-determined lean deposition rates were maximised during the first 4 weeks (0–4 weeks) of dietary CLA supplementation with no significant effects being observed in the subsequent 4 weeks (4–8 weeks) of the study. The DXA-determined lean tissue deposition rates were maximised at between 2·5 and 5·0 g CLA-55/kg diet, similar to that observed by Ostrowska *et al.* (1999). The chemical analyses in the present study, as well as our earlier work (Ostrowska *et al.* 1999), indicate that there was very little change in protein deposition, and that the change in lean tissue was due predominantly to an increase in water deposition. Since DXA technology estimates lean tissue from body water content, an increase in tissue hydration will be ascribed to an increase in lean tissue. Although there was no significant effect of CLA on water:protein in the present study, previously we have shown a significant increase in water:protein of a similar magnitude to that observed here (Ostrowska *et al.* 1999). Therefore, some care should be made in interpreting DXA-determined changes in body composition when there are also resultant changes in tissue hydration. It is also worth noting that the lean tissue deposition response to CLA supplementation was quadratic, being maximised at between 2·5 and 5·0 g CLA-55/kg, whereas the fat deposition response was linear. The different dose responses to CLA will need to be taken into account when determining the most cost-effective dose to feed on-farm.

The genotype used in the present study and that of Ostrowska *et al.* (1999) was of moderate fatness and gilts were utilised in these studies as they tend to deposit more fat than boars (Dunshea *et al.* 1993). However, the responses to CLA may vary with genetic propensity for fat or lean accretion. CLA response may also vary between gilts and barrows. For example, the benefits of a similar CLA preparation to that used in the present study appeared to be less pronounced in lean genotypes, as was observed in an on-farm study with boars and gilts (Dunshea *et al.* 2002). Also, the response varied between farms and/or genotypes and was greater in gilts than in boars (Dunshea *et al.* 2002). However, smaller but significant reductions in carcass P2 (−1·0 mm) and carcass fat (−7 g/kg) were recorded under commercial conditions at dietary CLA

concentrations as little as 2·2 g total CLA/kg. A recent short communication (Heckart *et al.* 2000) reported that feeding CLA decreased expression of fatty acid synthase in medium-lean line pigs and increased ($P<0·01$) expression in the high-lean pigs. The expression of stearoyl-CoA desaturase was decreased ($P<0·01$) in medium-lean line pigs and tended ($P=0·08$) to increase in the high-lean line group. These data suggest that CLA acts to modify expression of lipogenic genes in pigs that have not been selected for low fat deposition rates (Heckart *et al.* 2000). Furthermore, others have shown that supplementation of CLA does not alter backfat thickness in lactating sows (Harrell *et al.* 2000; Poulos *et al.* 2000). Therefore, it is possible that there are differences between the adolescent and adult animals and their response to CLA. There are data to suggest that CLA may affect growing animals differently from adults by depressing body fat accumulation via a reduction in preadipocyte number when given during periods of growth (Satory & Smith, 1999). In human studies, with weight-stable adults with stable body composition, no reduction in fat content was observed as a result of CLA (65 % purity) supplementation at 3 g/d (Medina *et al.* 2000; Zambell *et al.* 2000). However, a study with obese or overweight volunteers reported significant reductions in body fat at levels of 3·4 and 6·8 g CLA (75 % purity)/d (Blankson *et al.* 2000) and significant reductions in abdominal fat at 4 g CLA (75 % purity)/d (Riserus *et al.* 2001). If CLA does not stimulate lipolysis then one would expect to see no reduction in body fat already present in weight-stable adults. However, if it worked by inhibiting fat accretion, then CLA would work in adults providing they exercised or dieted so that the CLA could inhibit regain of fat. Data from our previous study in a pig model showed that the reduced fat accretion in pigs fed CLA was largely attributed to a reduced rate of lipogenesis from preformed fatty acids and possibly to a lesser extent due to increased lipolysis (Ostrowska *et al.* 2002).

The isomer thought to be responsible for the reduction in lipid accretion and storage in laboratory animals is the *trans*, *cis*-10,12; (de Deckere *et al.* 1999; Park *et al.* 1999*a*). This isomer was the most predominant in the CLA mixture used in the present study. The *trans*, *cis*-10,12 isomer was found to affect key enzymes involved in fatty acid uptake and transport. Some of the responses included a reduction in lipoprotein lipase activity, and lower levels of triacylglycerol and glycerol in adipocyte cells (Lin *et al.* 1999; Park *et al.* 1999*b*; Wang *et al.* 1999). Recently, the isomer was shown to markedly reduce the expression of genes encoding for two key enzymes, acetyl CoA carboxylase and fatty acid synthetase, involved in *de novo* fatty acid synthesis in dairy cows (Baumgard *et al.* 2002). Furthermore, the expression encoding for the enzyme that hydrolyses circulating triacylglycerol (lipoprotein lipase) as well as proteins involved in intracellular trafficking of fatty acids (fatty acid-binding protein) were also significantly reduced (Baumgard *et al.* 2002).

Further additional information gained from the present study was the effect of CLA on BMC and density. In recent studies with chicks and rats, CLA was shown to increase the rate of bone formation as indicated by an increase in percentage of ash in the body (Li & Watkins, 1998; Park & Pariza, 1998; de Deckere *et al.* 1999; Watkins & Seifert, 2000). In the present study, dietary CLA supplementation had no effect on the rate of ash deposition as determined by the slaughter-balance technique. The DXA estimates of bone density and content, both of which are free from assumptions regarding levels of tissue hydration (Haderslev *et al.* 1999), confirmed that CLA had little effect on these measures in pigs.

Despite widespread use of non-esterified fatty acid or methyl ester forms of CLA in research diets there is no evidence of the differences in the digestibility of different preparations. Non-esterified fatty acids are known to be less digestible in pigs when compared with triacylglycerols (Whittemore, 1993). Therefore the reduction in the feed intake in the CLA-fed pigs could be partially attributed to the acceptability of non-esterified fatty acids as lipid source. However, the growth rate was unaffected by CLA supplementation indicating that CLA non-esterified fatty acids were digested and metabolised sufficiently to maintain growth. Furthermore, the CLA was assimilated very efficiently into the tissue of pigs and in a dose-dependent manner (E Ostrowska, unpublished results). Today, the commercially available CLA is present in methyl esters of fatty acids. Lipids in this form are not found in natural diets and it is possible that esterified fatty acids could be less digestible than triacylglycerols or even non-esterified fatty acids. The ability of pigs to digest and assimilate these different forms of CLA needs to be more thoroughly investigated.

In conclusion, use of DXA technology has shown that the greatest reductions in fat deposition rates and increase in lean tissue deposition rates were achieved in the initial 4 weeks of CLA supplementation. This in turn suggests that the small additional benefits from long-term feeding with CLA may not necessarily be cost-effective. Additional factors including genetics, sex and age of the animal and the CLA isomer composition and its form probably play a vital role in determining the final outcome.

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