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Original article

## Effects of dietary conjugated linoleic acid on growth and body composition of control and IGF-I transgenic pigs<sup>1</sup>

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**Abstract** – Both dietary conjugated linoleic acid (CLA) and presence of the IGF-I transgene are reported to alter the fat:lean content of pigs. The purpose of this study was to compare the growth and body composition of control and IGF-I transgenic pigs in response to dietary CLA. Transgenic (TG) pigs expressing the IGF-I gene and sibling control (C) progeny were produced by mating two half-sib G-1 transgenic boars to non-transgenic gilts. At 60 kg each pig was scanned by dual-energy X-ray absorptiometry (DXA) for body composition analysis, then placed on an 18% crude protein diet containing either 2% corn oil (CO diet) or 1.2% CO plus 0.8% CLA. Thus, the four genotype-diet combinations were: C-CO (n=25), C-CLA (n=25), TG-CO (n=24), and TG-CLA (n=23). Each pig was scanned again at 110 kg. All pigs were slaughtered at 120 kg and the right half-carcass was scanned by DXA. Results of the DXA scan at 60 kg revealed that the TG pigs were less fat (15.0%) than the C pigs (18.8%,  $P < 0.05$ ). During growth from 60 to 110 kg, the tissue gain for the C-CO, C-CLA, TG-CO, and TG-CLA groups consisted of 16.6, 14.5, 13.2, and 13.0 kg of fat ( $P < 0.05$ , C-CO vs. C-CLA and C vs. TG); and 30.6, 32.6, 33.7, and 33.8 kg of lean ( $P < 0.05$ , C-CO vs. C-CLA and C vs. TG), respectively. There were only minor differences in bone growth. For the same groups, chemical analysis of the half-carcass revealed 29.2, 27.1, 23.8, and 22.8% fat ( $P < 0.05$  for C-CO vs. C-CLA and C vs. TG), respectively. Overall, the effects of CLA were less than those of TG on body or carcass composition; however, during the treatment period from 60 to 110 kg, the effects were similar. TG pigs did not respond to CLA as much as did control pigs.

**pig / CLA / IGF-I transgene / body composition**

**Résumé** – Effets de l'acide linoléique conjugué sur la croissance et la composition corporelle de porcs normaux et transgéniques IGF-I. L'acide linoléique conjugué (CLA) et la présence du

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transgène IGF-I ont tous deux des effets sur la composition corporelle du porc. L'objectif de cette étude était de comparer la croissance et la composition corporelle de porcs normaux et transgéniques IGF-I recevant dans leur ration du CLA. Des porcs transgéniques exprimant le gène IGF-I (TG) et des porcs témoins (C) ont été produits en croisant 2 verrats transgéniques de la même famille avec des truies normales. A 60 kg, chaque porc a été scanné par absorptiométrie biphotonique à rayons X (dual-energy X-ray absorptiometry, DXA) et a reçu une ration avec 18 % de matières azotées, contenant soit 2,0 % d'huile de maïs (CO), soit 1,2 % d'huile de maïs et 0,8 % de CLA. Ainsi 4 combinaisons génotypes  $\times$  rations ont été obtenues : C-CO (n=25), C-CLA (n=25), TG-CO (n=24) et TG-CLA (n=23). Chaque porc a été de nouveau scanné à 110 kg. Tous ont été abattus à 120 kg et leur demi-carresse droite scannée et analysée chimiquement. Les résultats de la scanographie faite à 60 kg montrent que les porcs TG avaient une teneur moindre en gras (15,0 %) que les porcs C (18,8 %,  $P < 0,05$ ). Durant la période de croissance de 60 à 110 kg, le gain tissulaire des groupes C-CO, C-CLA, TG-CO et TG-CLA se composait de 16,6, 14,5, 13,2 et 13,0 kg de gras ( $P < 0,05$ , C-CO vs. C-CLA et C vs. TG) et 30,6, 32,6, 33,7 et 33,8 kg de viande maigre ( $P < 0,05$ , C-CO vs. C-CLA et C vs. TG), respectivement. La croissance osseuse n'a montré que de faibles variations. Pour les mêmes groupes, la teneur en gras de la demi-carresse, mesurée par analyse chimique, était de 29,2, 27,1, 23,8 et 22,8 % ( $P < 0,05$ , C-CO vs. C-CLA et C vs. TG) respectivement. D'une manière générale, les effets du CLA ont été moins prononcés sur la composition corporelle et sur la carcasse que ceux dus à la présence du transgène IGF-I, mais ils ont été semblables durant la période expérimentale de 60 à 110 kg. Les porcs TG ont été moins sensibles à l'apport de CLA que les porcs témoins.

#### porc / CLA / transgène IGF-I / composition corporelle

### 1. INTRODUCTION

Numerous studies have been conducted to evaluate the potential of CLA (CLA, a mixture of positional and geometric conjugated diene isomers of octadecanoic acid) for improving growth performance and carcass composition of swine. Most of these studies have found that the addition of CLA to the diet results in some measure of fat reduction, however results have been inconsistent with regard to growth performance [4]. Supplementation of swine diets with CLA has been shown to decrease fat and increase lean tissue deposition [3, 5, 6, 19, 20, 27–30]. While several studies reported that CLA increased the gain:feed ratio [5, 6, 19, 20, 28–30], only one reported an increase in daily gain [26]. Most of these studies relied on end point measures based on carcass dissection or linear measures of backfat and loin eye area. In order to measure the changes in composition during the period of CLA supplementation, Ostrowska et al. [18] used comparative slaughter to determine body composition at the beginning and end of the study. Dual energy X-ray absorptiometry (DXA) offers an

alternative to comparative slaughter for measuring changes in body composition in the live animal. DXA has been shown to be useful for evaluating the composition of both the live pig [7, 12–16, 18, 20, 25, 26] and the pork carcass [17, 24]. Using DXA, Ostrowska et al. [20] measured the temporal and dose-dependent effects of CLA on body composition of pigs.

Little information is available with respect to how diverse genotypes of pigs respond to dietary CLA. Dugan et al. [5] reported that gilts and barrows respond similarly to CLA supplementation. Wiegand et al. [29] found no interaction between the stress-genotype status of pigs and the subsequent effect of CLA on their growth and performance. Pigs expressing the IGF-I transgene have less fat and more lean tissue than their littermate controls [21]. In these pigs, the IGF-I gene is expressed directly in the skeletal muscle and there is no elevation in circulating GH [21]. In question in the present study was whether or not supplemental CLA would result in a further improvement in body composition (less fat and more lean) in pigs expressing the IGF-I transgene. Therefore, the purpose of this study was to compare the

growth and body composition of control and IGF-I transgenic pigs in response to dietary CLA during growth from 60 to 110 kg body weight (BW) using DXA to make live animal and carcass composition measurements.

## 2. MATERIALS AND METHODS

### 2.1. Animals and diets

Transgenic pigs were produced with a fusion gene composed of avian skeletal – actin regulatory sequences and the cDNA encoding IGF-I [21]. Transgenic (TG) and sibling control (C) progeny were produced by mating two half-sibling G-1 transgenic boars to non-transgenic gilts. Presence of the IGF-I transgene was established by Southern blot hybridization analysis of DNA from tail biopsies of the G1 progeny taken at birth. At 60 kg, the pigs were placed on diets with a CP content of 182 g·kg<sup>-1</sup>, a ME content of 13.8 MJ·kg<sup>-1</sup>, and supplemented with either corn oil at 20 g·kg<sup>-1</sup> (CO) or CO at 12 g·kg<sup>-1</sup> plus CLA at 8 g·kg<sup>-1</sup> (CLA). A total of 97 pigs were used in two replicates of a 2 × 2 × 2 factorial arrangement, with the respective factors being diet (CO or CLA), genotype (TG or C), and sex (female or barrow). When possible, a non-transgenic control littermate of the same sex was paired with each transgenic pig and the pair was assigned randomly to their respective diet. Thus, the four genotype-diet combinations were: C-CO (n=25), C-CLA (n=25), TG-CO (n=24), and TG-CLA (n=23). Distributed among these were 54 gilts and 43 barrows. Total feed intake for the 60 to 110 kg growth period was measured for each pig individually. The pigs were housed in individual pens and hand-fed daily with the amount of feed offered based on body weight and adjusted each week after the pigs were weighed. The amount fed was 110% of calculated ad libitum energy intake [1]. The pigs were weighed weekly on an electronic scale (+0.2 kg) and the amount of feed presented was adjusted

**Table I.** Ingredient composition (wt-%, as-fed basis) of diets<sup>A</sup>.

Item	Treatment	
	CO	CLA
Corn	61.80	61.80
Soybean meal (48% CP)	18.00	18.00
Dry skim milk, non-fat	12.00	12.00
L-Lysine HCl	0.25	0.25
Sodium monophosphate	2.50	2.50
Calcium carbonate	2.50	2.50
NaCl	0.50	0.50
Selenium premix <sup>B</sup>	0.05	0.05
Mineral premix <sup>C</sup>	0.20	0.20
Vitamin premix <sup>D</sup>	0.20	0.20
Corn oil	2.00	1.20
Conjugated linoleic acid (75%) <sup>E</sup>	0.00	0.80

<sup>A</sup> Calculated nutrient composition: crude protein 18%; ME 13.8 MJ per kg; and total lysine 1.2% (0.87 g per MJ ME).

<sup>B</sup> Supplied per kg of diet: 100 µg selenium as sodium selenite.

<sup>C</sup> Provided the following micronutrients in mg per kg complete diet: 100 Mn, 100 Fe, 10 Cu, 1 Co, 3 I, and 100 Zn.

<sup>D</sup> Supplied per kg complete diet: 4 400 IU vitamin A, 800 IU vitamin D, 11 IU vitamin E, 8.8 mg riboflavin, 17.6 mg pantothenic acid, 30.8 mg niacin, 44 µg vitamin B<sub>12</sub>, and 220 mg choline.

<sup>E</sup> CLA contained the following (of total fatty acids based on manufacturers GC analysis, Pharmedics, Lake Bluff, IL): C16:0 (palmitic acid), 4.4%; C18:0 (stearic acid), 2.8%; C18:1 (oleic acid), 15.4%; C18:2 c9, c12 (linoleic acid), 1.8%; and C18:2, conjugated (CLA), consisting of 34.9% c9,t11 and 35.9% t10,c12 isomers.

according to body weight. Feed not consumed each day was weighed and discarded. Composition of the experimental diets is shown in Table I. The basal diet used in this study is the same as used in previous studies with this population of IGF-I TG pigs [21, 22]. The CLA supplement was a free fatty acid oil consisting of an isomeric form of octadecadienoic acid (linoleic acid) containing approximately equal portions of the c9, t11 and t10, c12 isomers. Approximately 75% of the product was CLA (Tab. I).

Water was available ad libitum. The experimental protocols used in this research were approved by the Beltsville Area Institutional Animal Care and Use Committee.

## 2.2. Live animal measurements

Each pig was scanned at 60 and again at 110 kg BW by dual-energy X-ray absorptiometry (DXA) for body composition analysis using a Lunar DPX-L instrument (GE-Lunar, Madison, WI) as described by Mitchell et al. [7]. Pigs were fasted overnight and then anesthetized using a mixture of Ketamine, Tiletamine, Zolazepam, and Zylazine [13] to prevent movement during the scanning procedure. The DXA scans provided measurements of total body fat, lean, bone mineral content (BMC), and bone mineral density (BMD). Total body fat was calculated from the DXA readings using the following equation from Mitchell et al. [15]:  $\text{Fat (\%)} = 493.4 + (\text{DXA R value} \times 348.8)$ . Bone content was calculated from DXA bone mineral (BMC) values ( $\text{Bone (kg)} = \text{BMC} \times 4.14$ ) based on the 24.14% ash content of pork bones reported by Field et al. [10]. Lean content (including internal organs, connective tissue and gut fill) was calculated as:  $\text{Lean (kg)} = \text{BW} - (\text{Fat} + \text{Bone})$ . DXA measurements of bone mineral density (BMD, g per  $\text{cm}^2$ ) were made for various regions of the body as described by Mitchell et al. [16]. Ultrasound measurements (Aloca Model SSD-500V) of loin eye area (LEA) and backfat (BF) depth were made at the level of the 10th rib while the pigs were positioned for scanning on the DXA instrument. Total body fat, lean and bone deposition were based on the differences between the 60 and 110-kg DXA measurements of fat, lean and BMC.

## 2.3. Carcass measurements

All pigs were slaughtered at  $122 \pm 3$  kg BW (mean  $\pm$  SD) and the right half-carcass was scanned by DXA [17]. DXA measurements of fat content were adjusted using the following formulas: TOTAL CARCASS %

FAT =  $450 - (315 \times \text{R value})$ ; SHOULDER % FAT =  $390 - (269 \times \text{R value})$ ; HAM % FAT =  $356 - (245 \times \text{R value})$ ; LOIN % FAT =  $450 - (315 \times \text{R value})$ ; BELLY % FAT =  $439 - (298 \times \text{R value})$ . Bone content was calculated as described above for the total body. Lean content was calculated as:  $\text{Lean} = \text{CW} - (\text{Fat} + \text{Bone})$ , where CW is the carcass weight. In addition, the right half-carcass was analyzed chemically for lipid [11], protein (Kjeldahl nitrogen), water (lyophilization), and ash content (muffle furnace, 24 h at 500 °C) as described by Mitchell et al. [13].

## 2.4. Plasma pGH and IGF-I

Blood was collected from the ear vein under anesthesia during the DXA scanning at 60 and 110 kg BW, and during exsanguination when the pigs were sacrificed at 120 kg BW. At the time of blood collection, all pigs had been fasted about 20 h at 60 and 110 kg BW and fasted about 44 h when sacrificed. Blood was collected into a syringe that contained EDTA to prevent coagulation, and plasma was separated by centrifugation and stored at  $-70$  °C until assay. Plasma concentrations of pGH were determined by radioimmunoassay using guinea pig anti-porcine GH serum [23]. Plasma IGF-I was measured by double antibody radioimmunoassay using rabbit anti-human IGF-I (UBK-487, NIDDK, Bethesda, MD) as the primary antiserum and recombinant human IGF-I for standard and iodination tracer (R-D Systems, Inc., Minneapolis, MN). Samples were prepared for assay using the acid glycyglycine acidification technique [9]. The samples were all evaluated in a single assay. The intra-assay coefficient of variation was 5.8%.

## 2.5. Statistical analysis

Differences in growth and body or carcass composition were analyzed by the GLM procedure of SAS (version 8.2) for a factorial design with the main treatment effects being diet, genotype and sex. Two

and three way interactions were evaluated for each treatment combination. The experimental unit for all measures was the individual pig. SAS analysis was performed at the BARC Biometrical Consulting Service. Other statistical analysis were performed in-house using Statgraphics® Plus for Windows 2.1. Differences in plasma IGF-I and pGH and differences among gene-diet groups were analyzed by ANOVA. Chemical and DXA measurements of carcass composition were compared by *t*-test and regression analysis.

### 3. RESULTS

#### 3.1. Measurements of in vivo body composition at 60 and 110 kg body weight

At the initial measurement at 60 kg, the IGF-I transgenic pigs were found to have 20% less fat, 6% more lean tissue, 9% larger LEA and 16% less BF than the control pigs (Tab. II). The measurements at 110 kg BW revealed that similar differences persisted between the TG and C pigs. At 110 kg BW, pigs fed the CLA diet had 6% larger LEA than pigs fed the control diet. At both 60 and 110 kg BW, gilts had less fat, more lean, larger LEA, and less BF than did barrows. At 110 kg, the gilts had less bone than did barrows. There were no gene  $\times$  sex or diet  $\times$  sex interactions. At 110 kg BW, there were significant gene  $\times$  diet  $\times$  sex interactions for body composition (fat and lean content, LEA, and BF).

The only gene  $\times$  diet interaction was for BW at 110 kg. For the gene-diet groupings, at 110 kg BW the C-CLA pigs had less fat, more lean tissue and larger LEA ( $P < 0.05$  for each) compared to C-CO pigs. At 110 kg BW no ( $P > 0.05$ ) differences were observed between TG-C and TG-CLA pigs. At 60 kg BW, the TG-CO group had lower ( $P < 0.05$ ) bone mass and bone density (BMD) compared to the C-CO group. At 110 kg BW, there was no ( $P > 0.05$ ) differ-

ence in total body bone mass, however, the TG-CO pigs had lower BMD than either of the control groups (C-CO and C-CLA).

#### 3.2. Plasma IGF-I and pGH

The mean plasma concentrations of IGF-I and GH measured at 60 BW, 110 and 120 kg BW are shown in Figure 1. Concentrations of plasma IGF-I were higher ( $P < 0.05$ ) in TG pigs at each time period. Overall, the level of plasma IGF-I was 21% higher ( $P < 0.0001$ ) in the TG pigs compared to the C pigs. There was no ( $P > 0.05$ ) CLA effect on plasma IGF-I concentration; however, the levels in gilts were higher (128 ng per mL) than in barrows (116 ng per mL,  $P = 0.005$ ). At 60 kg BW there was no ( $P = 0.71$ ) difference, but at 110 and 120 kg BW, the plasma IGF-I was 12% (129 vs. 115 ng per mL) and 15% higher (138 vs. 120 ng per mL), respectively, in gilts than in barrows ( $P = 0.03$ ). There were no ( $P > 0.05$ ) differences in plasma GH concentrations regardless of gender, diet or, transgene.

#### 3.3. Rate and composition of growth between 60 and 110 kg body weight

During the period of growth from 60 to 110 kg BW, the TG pigs had a higher average daily gain (ADG) and feed efficiency (G/F) ( $P = 0.002$  and  $P = 0.009$ ), compared to the C pigs (Tab. III). The TG pigs deposited 2.6 kg less fat as the result of a 17% reduction in the rate of fat deposition. At the same time, the TG pigs had a 14% increase in the rate of lean deposition resulting in 1.6 kg more total body lean tissue. The combined effect was a 20% reduction in the fat:lean gain in TG pigs. The addition of CLA to the diet resulted in an overall 10% reduction in the rate of fat deposition during growth from 60 to 110 kg. The pigs receiving CLA deposited 5% more lean tissue. Consequently, CLA supplementation resulted in a 12% reduction in the fat:lean gain. Compared to barrows, gilts had a lower rate of fat deposition and a higher rate of lean deposition resulting in less fat and more

**Table II.** Effect of CLA and IGF-I transgene on body composition measurements of live pigs<sup>A</sup>.

Measurement	Treatments								Interactions							
	Control pigs				IGF transgenic pigs				Main effects			Gene × diet × sex				RMS <sup>B</sup>
	Control diet		CLA diet		Control diet		CLA diet					Gene	Gene	Diet	Diet	
	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gene	Diet	Sex	Gene	Diet	Sex		
<i>Initial (60 kg)</i>																
BW (kg)	59.8	59.5	60.1	60.4	61.5	60.5	60.2	60.4	NS <sup>C</sup>	----F	NS	----F	NS	----F	----F	2.19
Fat <sup>D</sup> (kg)	11.4	11.2	10.8	11.6	8.0	9.9	9.2	9.1	<0.0001	---	0.03	---	NS	---	---	1.34
Lean <sup>D</sup> (kg)	42.4	42.5	43.2	42.1	46.2	44.1	44.7	45.0	<0.0001	---	0.03	---	NS	---	---	1.52
Bone <sup>D</sup> (kg)	6.18	6.22	5.98	6.24	5.80	5.98	6.04	5.94	0.02	---	NS	---	NS	---	---	0.46
BMD (g per cm <sup>2</sup> )	1.00	0.99	0.97	1.00	0.95	0.98	0.99	0.97	NS	---	NS	---	NS	---	---	0.05
LEA (cm <sup>2</sup> )	21.3	20.5	22.7	19.7	25.1	20.8	23.9	21.9	0.0008	---	<0.0001	---	NS	---	---	2.63
BF (cm)	1.84	1.84	1.71	1.82	1.39	1.72	1.46	1.58	<0.0001	---	0.03	---	NS	---	---	0.30
<i>Final (110 kg)</i>																
BW (kg)	110.7	110.4	110.2	110.6	109.8	109.2	111.2	110.9	NS	NS	NS	NS	NS	NS	NS	2.00
Fat <sup>E</sup> (kg)	28.0	27.9	24.3	27.3	19.7	24.9	21.4	23.0	<0.0001	NS	0.0009	NS	NS	NS	0.02	3.46
Lean <sup>E</sup> (kg)	73.2	72.9	76.8	73.6	81.5	75.9	79.5	77.7	<0.0001	NS	0.0003	NS	NS	NS	0.02	3.50
Bone <sup>E</sup> (kg)	8.76	9.19	8.95	9.13	8.84	9.19	9.07	9.31	NS	NS	0.04	NS	NS	NS	NS	0.69
BMD (g per cm <sup>2</sup> )	1.18	1.19	1.19	1.21	1.15	1.15	1.18	1.17	0.006	NS	NS	NS	NS	NS	NS	0.05
LEA (cm <sup>2</sup> )	35.1	32.8	38.6	34.1	39.2	34.5	40.4	37.1	0.01	0.03	0.0003	NS	NS	NS	0.02	4.84
BF (cm)	2.98	3.03	2.67	3.01	1.88	2.70	2.33	2.63	<0.0001	NS	0.0004	NS	NS	NS	0.05	0.50

<sup>A</sup> Body weight (BW) measured by electronic scale weighing (+0.1 kg); fat, lean and bone estimated from total body DXA scans where Fat (%) = 493.4 + (DXA R value × 348.8), Bone = BMC × 4.14, and Lean = BW – (Fat + Bone); loin eye area (LEA) and backfat (BF) measured at 10th rib by ultrasound; BMD: bone mineral density.

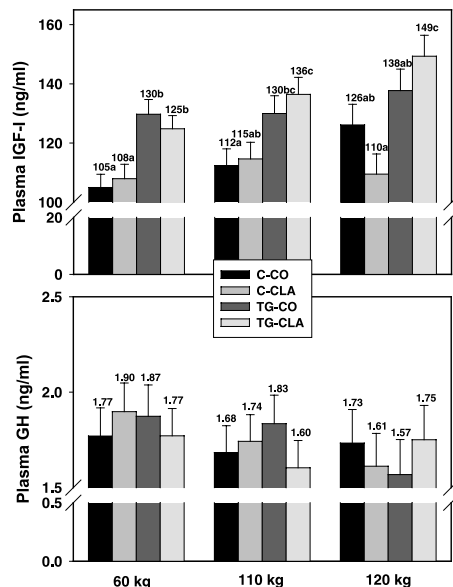
<sup>B</sup> Root Mean Square Error (RMS).

<sup>C</sup> NS, *P*-value > 0.05.

<sup>D</sup> Values were adjusted to 60 kg BW.

<sup>E</sup> Values were adjusted to 110 kg BW.

<sup>F</sup> All pigs were fed the same control diet prior to 60 kg BW.



**Figure 1.** Least squares means of plasma concentrations of IGF-I and pGH for control (C) and transgenic (TG) pigs fed control diet (CO) or diet supplemented with conjugated linoleic acid (CLA). Blood samples from each pig were collected at 60, 110, and 120 kg body weight. Vertical bars are standard errors of the mean, and mean values are above each bar. Within each weight group, means followed by a different letter were significantly different ( $P < 0.05$ ).

lean being deposited, hence a lower fat:lean gain. The TG pigs had a 19% higher rate of bone deposition. Neither diet nor sex had an effect on bone deposition and none of the treatment variables resulted in changes in total body BMD. There were no gene  $\times$  sex or gene  $\times$  diet  $\times$  sex interactions and the only diet  $\times$  sex interaction was for feed intake.

Significant gene  $\times$  diet interactions were noted for fat gain (fat kg per day and BF). Figure 2 shows the rates of fat, lean and bone growth that was measured in the four gene-diet groups as they grew from 60 to 110 kg BW. During this period, the TG-CO pigs deposited 20% less fat and 10% more

lean tissue compared to their littermate controls (C) fed the CO diet which corresponded to a 15% lower fat and a 16% higher lean tissue deposition rate ( $P < 0.05$ ). Pigs of the C-CLA group deposited 13% less fat than the C-CO pigs and the fat deposition rate was 15% lower ( $P < 0.05$ ). Although the C-CLA pigs deposited 6.5% more lean, the rate of deposition was not different ( $P > 0.05$ ) from that of the C-CO pigs. CLA did not have a significant effect on fat and lean deposition in the TG pigs.

Based on ultrasound measurements at 60 and 110 kg BW, the largest increase in LEA was in the pigs fed the CLA diets ( $P = 0.01$ ). There was an 18% greater ( $P < 0.05$ ) increase in LEA in C-CLA pigs and a non-significant 13% greater ( $P > 0.05$ ) increase in TG-CLA pigs compared to their respective groups fed the CO diet. The increase in BF was 26% less in TG pigs compared to controls ( $P = 0.0004$ ). The increase in BF was 41% less ( $P < 0.05$ ) in the TG-CO pigs than in C-CLA pigs. There was also a smaller increase in BF in C-CLA pigs (not significant,  $P > 0.05$ ). However, a 35% greater ( $P < 0.05$ ) increase in BF was measured in the TG-CLA pigs compared to the TG-CO pigs.

### 3.4. DXA measurements of changes in composition of body regions

The DXA measurements of fat, lean and bone content of each pig at 60 and 110 kg BW were analyzed for three major body regions: front legs, trunk and back legs. The calculated growth of each tissue component within these regions is shown in Table IV. TG pigs gained significantly less fat and more lean in all three regions. Only in the trunk region did the TG pigs have increased deposition of bone mineral (BMC). Pigs receiving CLA deposited less fat in the front and back leg regions and more lean in all three regions. Gilts gained less fat than barrows in all three regions, more lean in the trunk region, and more BMC in the back legs. There were few differences in regional bone densities (data not shown), pigs fed



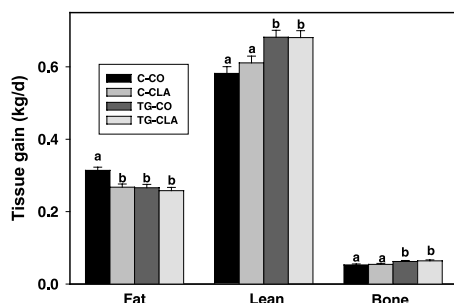
**Table III.** Influence of CLA and IGF-I transgene on the growth of pigs between 60 and 110 kg body weight<sup>A</sup>.

Measurement	Treatments						Interactions					
	Control pigs			IGF transgenic pigs			Main effects			Gene × diet × sex		
	Control diet		CLA diet	Control diet		CLA diet	Gene	Diet	Sex	Gene × diet × sex	Gene × diet × sex	Gene × diet × sex
	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow						
ADG (kg per d)	0.94	0.96	0.93	0.94	1.03	0.99	1.02	0.99	NS	NS	NS	NS
Feed (kg per d)	3.01	3.07	3.03	3.03	2.86	3.10	3.07	3.01	NS	NS	NS	NS
Gain/feed	0.31	0.32	0.31	0.31	0.36	0.32	0.33	0.33	NS	NS	NS	NS
Fat (kg per d)	0.31	0.32	0.25	0.29	0.24	0.30	0.25	0.27	0.002	0.001	0.0003	0.04
Lean (kg per d)	0.58	0.59	0.62	0.60	0.72	0.63	0.71	0.65	<0.0001	NS	NS	NS
Fat:lean gain	0.55	0.55	0.40	0.50	0.34	0.49	0.36	0.44	<0.0001	0.02	0.0006	NS
Bone (g per d)	49.44	57.78	54.74	53.97	61.60	63.11	61.71	66.50	0.0007	NS	NS	NS
BMD (g per cm <sup>2</sup> )	0.18	0.20	0.22	0.22	0.20	0.18	0.19	0.20	NS	NS	NS	NS
LEA (cm <sup>2</sup> )	13.62	12.38	15.92	14.36	14.08	13.67	16.35	15.20	NS	0.01	NS	NS
BF (cm)	1.16	1.18	0.96	1.18	0.48	0.98	0.88	1.05	0.0004	NS	0.003	NS

<sup>A</sup> Fat, lean and bone estimated from total body DXA scans where Fat (%) = 493.4 + (DXA R value × 348.8), Bone (kg) = BMC × 4.14, and Lean (kg) = BW - (Fat + Bone); loin eye area (LEA) and backfat (BF) measured at 10th rib by ultrasound; ADG: average daily gain; BMD: bone mineral density.

<sup>B</sup> Root Mean Square Error (RMS).

<sup>C</sup> NS, *P*-value > 0.05.



**Figure 2.** Fat, lean, and bone gain as measured by DXA for the period of body growth between 60 and 110 kg body weight in control (C) and IGF-transgenic (TG) pigs fed either the control (CO) or the CLA supplemented diet. Within each tissue type, different letters above bars indicate significant differences ( $P < 0.05$ ).

the CLA diet had higher bone density in the front legs ( $P = 0.01$ ) and back legs ( $P = 0.04$ ).

The only significant interactions were for gene  $\times$  diet for fat and BMC gain in the trunk region. Comparing the gene-diet groups, the TG-CO pigs gained less ( $P < 0.05$ ) fat and more ( $P < 0.05$ ) lean in each region when compared to the C-CO pigs. The greatest difference was in the trunk region where the TG pigs gained 41% less fat and 19% more lean ( $P < 0.05$  for each). Compared to C-CO pigs, pigs of the C-CLA group gained less fat and more lean in all regions, although the difference was not significant for fat growth in the trunk region and lean growth in the front leg region. The only difference in bone growth was a greater ( $P < 0.05$ ) bone growth in the trunk region of the TG-CLA pigs compared to the other groups.

### 3.5. DXA and chemical measurements of carcass composition

The results of DXA and chemical analysis of the half-carcass of pigs slaughtered at 122 kg BW are shown in Table V. Consistent with the live DXA measurements at 110 kg BW, DXA analysis of the carcasses

of the TG pig revealed less fat and more lean than those of the control pigs. Likewise, chemical analysis found less fat, higher water content, a higher percentage of protein in the carcasses of the TG pigs compared to controls. Pigs that had received CLA had less carcass fat based on both DXA and chemical analysis. The carcasses of the gilts had less fat and more lean (protein and water by chemical analysis) than barrows by both DXA and chemical analysis. There were no gene  $\times$  diet or diet  $\times$  sex interactions and the only significant gene  $\times$  sex interaction was for carcass fat percentage by chemical analysis. Significant gene  $\times$  diet  $\times$  sex interactions were noted for DXA measured percentages of carcass fat and lean and chemical measurement of percentage of protein in the carcass.

The DXA measurements of the half-carcass by regions are shown in Table VI. The TG pigs had less fat, a lower percentage of fat, and a higher percentage of lean in all four carcass regions. Pigs fed the CLA diet had a lower percentage of fat in the shoulder and ham regions. Compared to barrows, gilts had less fat and more lean in all regions of the carcass. Barrows had more bone in the ham region, but less in the side region. There were no significant diet  $\times$  sex, gene  $\times$  sex, or gene  $\times$  diet interactions for any region. In each region there were significant gene  $\times$  diet  $\times$  sex interactions for the percentages of fat and lean.

A comparison of DXA and chemical analysis for measurement of composition of the half-carcass is shown in Table VII. There was good agreement between DXA and chemical determinations of carcass fat content with nearly identical mean values and high regression coefficients ( $R^2$ ) for both kg of fat and percentage of fat. The DXA measurements of lean mass were significantly higher (3%) than the chemical lean values calculated by combining protein and water measurements, however the  $R^2$  values were high. The DXA BMC measurement was significantly higher (17%)

**Table IV.** Effects of CLA and IGF-I transgene on the deposition of fat, lean and bone mineral (BMC) in different body regions of pigs during growth between 60 and 110 kg body weight<sup>A</sup>.

Measurement	Treatments						Interactions						RMS <sup>B</sup>		
	Control pigs			IGF transgenic pigs			Main effects			Gene × diet × sex					
	Control diet		CLA diet	Control diet		CLA diet	Gene	Diet	Sex	Gene × diet	Diet × sex	Gene × diet × sex			
	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow									
<i>Fat (kg)</i>															
Front legs	4.21	4.39	3.24	3.94	2.81	3.58	2.71	3.32	0.0004	0.05	0.01	NS <sup>C</sup>	NS	NS	1.10
Trunk	7.02	6.17	4.91	6.06	2.77	5.10	4.15	5.38	0.0008	NS	0.05	0.05	NS	NS	2.36
Back Legs	4.05	3.97	3.17	3.66	2.41	3.28	2.47	2.66	<0.0001	0.01	0.03	NS	NS	NS	0.83
<i>Lean (kg)</i>															
Front legs	4.86	4.64	5.30	5.14	5.52	4.94	5.92	5.67	0.004	0.005	NS	NS	NS	NS	0.87
Trunk	16.93	14.86	19.23	16.95	20.22	17.63	20.77	19.22	0.0007	0.02	0.003	NS	NS	NS	3.40
Back Legs	7.98	8.39	8.97	8.77	9.36	8.54	9.48	9.13	0.01	0.03	NS	NS	NS	NS	1.15
<i>BMC (kg)</i>															
Front legs	0.02	0.23	0.23	0.22	0.22	0.24	0.21	0.23	NS	NS	NS	NS	NS	NS	0.049
Trunk	0.07	0.09	0.08	0.05	0.08	0.09	0.12	0.17	0.007	NS	NS	0.01	NS	NS	0.076
Back Legs	0.22	0.25	0.25	0.26	0.22	0.26	0.23	0.26	NS	NS	0.005	NS	NS	NS	0.047

<sup>A</sup> Fat, lean, and BMC measurements were based on region-of-interest analysis of body DXA scans without any adjustments.<sup>B</sup> Root Mean Square Error (RMS).<sup>C</sup> NS, *P*-value > 0.05.

**Table V.** Effect of CLA and IGF-I transgene on the carcass composition of pigs as measured by DXA or chemical analysis<sup>A</sup>.

Measurement	Treatments						Interactions								
	Control pigs			IGF transgenic pigs			Main effects			Gene × Diet × Gene × diet × sex					
	Control diet		CLA diet	Control diet		CLA diet	Gene		Diet	Sex	Gene × diet × sex				
	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gilt	Barrow	Gene	Diet	Sex	RMS <sup>B</sup>			
<i>1/2</i> CW (kg)	45.6	45.1	44.8	44.2	43.9	43.8	43.3	42.9	0.0006	NS <sup>C</sup>	NS	NS	NS	NS	1.94
<i>DXA</i>															
Fat (%)	27.98	28.59	26.03	27.95	22.20	27.27	22.96	24.40	<0.0001	0.05	0.0003	NS	NS	NS	2.91
Lean (%)	61.16	60.13	62.80	60.70	67.07	61.58	66.22	64.83	<0.0001	NS	0.0002	NS	NS	NS	3.09
Bone (%)	10.86	11.09	11.16	11.35	10.72	11.15	10.82	10.77	NS	NS	NS	NS	NS	NS	0.90
<i>Chemical</i>															
Fat (%)	28.94	29.56	26.01	28.37	21.59	26.48	21.13	24.97	<0.0001	0.04	0.0002	NS	0.05	NS	3.57
Protein (%)	16.21	15.76	16.84	15.71	17.11	15.82	16.71	17.02	0.04	NS	0.01	NS	NS	NS	1.21
Water (%)	50.33	50.07	52.77	51.10	56.69	52.10	56.49	54.30	<0.0001	0.05	0.002	NS	NS	NS	3.35
Ash (%)	2.63	2.94	2.77	2.82	3.11	2.78	2.90	2.96	NS	NS	NS	NS	NS	NS	0.44

<sup>A</sup> Half-carcass weight (*1/2*CW) measured by electronic scale weighing (+0.1 kg); DXA fat, lean and bone estimated from DXA scans of entire half-carcass where Fat (%) =  $450 - (315 \times R \text{ value})$ , Bone (kg) =  $\frac{1}{2}\text{CW} - (\text{Fat} + \text{Bone})$ ; chemical analysis of fat, protein, water and ash for entire half-carcass.

<sup>B</sup> Root Mean Square Error (RMS).

<sup>C</sup> NS, *P*-value > 0.05.

**Table VI.** Effects of CLA and IGF-I transgene on the percentages of fat, lean and bone in regions of the half-carcass of pigs<sup>A</sup>.

Measurement	Treatments						Interactions					
	Control pigs			IGF transgenic pigs			Main effects			Gene × diet × sex		
	Control diet		CLA diet	Control diet		CLA diet	Gene		Diet	Gene × diet × sex		RMS <sup>B</sup>
	Gilt	Barrow		Gilt	Barrow		Gilt	Barrow	Sex	Gene	Diet	sex
<i>Shoulder</i>												
Fat (%)	29.13	29.67	27.28	29.14	24.47	28.76	24.86	26.39	<0.0001	0.03	<0.0001	NS <sup>C</sup>
Lean (%)	56.06	55.40	57.67	56.04	61.63	56.10	60.79	59.34	0.0001	NS	0.0003	NS
Bone (%)	14.81	14.92	15.04	14.82	13.90	15.14	14.34	14.27	NS	NS	NS	NS
<i>Loin</i>												
Fat (%)	27.44	28.40	25.33	27.69	21.42	26.58	22.30	23.99	<0.0001	NS	<0.0001	NS
Lean (%)	62.62	61.52	64.58	61.47	68.24	63.81	67.68	66.72	<0.0001	NS	0.0004	NS
Bone (%)	9.94	10.07	10.09	10.84	10.34	9.61	10.01	9.28	NS	NS	NS	NS
<i>Side</i>												
Fat (%)	41.90	42.21	40.39	42.14	36.70	40.93	37.91	38.49	<0.0001	NS	0.005	NS
Lean (%)	56.82	56.37	57.88	56.47	61.15	57.53	60.10	59.87	<0.0001	NS	0.0003	NS
Bone (%)	1.28	1.42	1.73	1.39	2.15	1.54	1.99	1.65	0.002	NS	0.02	NS
<i>Ham</i>												
Fat (%)	27.82	28.25	25.79	27.64	21.86	26.86	22.47	23.74	<0.0001	0.05	0.001	NS
Lean (%)	58.68	57.90	60.16	57.91	63.73	58.82	63.09	61.65	<0.0001	NS	0.0001	NS
Bone (%)	13.68	14.13	13.78	14.59	13.27	14.29	13.43	13.89	NS	NS	0.002	NS

<sup>A</sup> DXA measurements of fat, lean and bone estimated from region-of-interest analysis half-carcass scans: SHOULDER % FAT = 390 – (269 × R value); HAM % FAT = 356 – (245 × R value); LOIN % FAT = 450 – (315 × R value); BELLY % FAT = 439 – (298 × R value); Bone (kg) = BMC × 4.14; and Lean (kg) = region WT – (Fat + Bone), WT: weight.

<sup>B</sup> Root Mean Square Error (RMS).

<sup>C</sup> NS, *P*-value > 0.05.

**Table VII.** Comparison of DXA and chemical measurements of carcass composition (n=97).

Measurement	DXA <sup>A</sup>	Chemical <sup>A</sup>	<i>P</i> -value <sup>B</sup>	R <sup>2</sup>	SEE <sup>C</sup>
Fat (kg)	11.49 ± 1.89	11.47 ± 2.26	0.94	0.85	0.74
Fat (%)	25.91 ± 3.63	25.86 ± 4.60	0.94	0.81	1.57
Lean (kg)	31.58 ± 1.73 <sup>D</sup>	30.67 ± 2.15 <sup>E</sup>	0.002	0.88	0.59
Lean (%)	71.44 ± 3.67	69.40 ± 4.71	0.001	0.86	1.44
BMC/ash (kg)	1.17 ± 0.11	1.00 ± 0.21 <sup>F</sup>	<0.0001	0.11	0.20

<sup>A</sup> DXA or chemical value ± SD.<sup>B</sup> *P* value for comparison of DXA and chemical means.<sup>C</sup> Standard error of estimate for linear regression.<sup>D</sup> DXA lean calculated as Lean = CW – (DXA fat + DXA BMC).<sup>E</sup> Chemical lean calculated as Lean = protein + water.<sup>F</sup> Chemical bone ash values were calculated as Ash = carcass ash – (lean mass × 0.0085).

than the chemical bone ash value (ash corrected for lean tissue mineral content) and there was a low correlation ( $R^2$ ) between the two measurements. All of the chemical measurements had higher standard deviations than the DXA measurements.

#### 4. DISCUSSION

The IGF-I transgenic pigs used in this study were derived from a population that has been described in previous studies [21, 22]. The differences in body composition between control and IGF-I transgenic pigs, wherein the TG pigs had less fat and more lean at both 60 and 110 kg BW, are similar to those reported previously. Consistent with the total body measurements, the TG pigs had less BF and larger LEA compared to the C pigs. Likewise, the plasma IGF-I concentrations observed in this study are similar to those in a previous study [21] that reported slightly higher levels in transgenic compared to non-transgenic pigs, and also, higher levels in boars compared to gilts in both non-transgenic and transgenic pigs. As with the previous study, there was no difference in the level of plasma GH between the TG and C pigs, indicating that the slight elevation in circulating IGF-I was not enough to suppress plasma GH in the TG pigs. The TG pigs had a higher ADG and

feed efficiency compared to the C pigs during the growth period from 60 to 110 kg BW. This is in contrast to the lack of an effect of the IGF transgene on the rate of gain and feed efficiency of gilts and boars reported in previous studies [21, 22]. Perhaps contributing to the improvement in growth performance was the significant increase in lean tissue deposition in the TG pigs compared to C pigs that was not observed in the prior studies.

##### 4.1. Effects of CLA

The addition of CLA to the diet did not affect either ADG or feed efficiency. The lack of an effect of CLA on ADG is in agreement with other studies, however the same studies reported an increase in G/F [6, 19, 20, 29, 30]. The improvement in feed efficiency has been attributed to a decrease in feed intake [20]. No differences in feed intake were observed in the present study, however it should be noted that all pigs were fed at 110% of calculated ad libitum intake rather than true ad libitum. The primary effects of CLA that were observed in this study were a lower rate of fat deposition and a lower fat:lean gain during the period of growth between 60 and 110 kg BW. The reduction in fat deposition resulted in less fat in the carcass, but no difference in the

DXA measurement of total body fat or the ultrasound measured BF at 110 kg BW. Numerous other studies have reported that feeding CLA to swine resulted in a reduction in BF [3, 5, 6, 28–30] or fat mass [6, 19, 20, 27]. The only indications of increased lean as the result of CLA were a larger LEA (live ultrasound at 110 kg BW) and a higher percentage of water in the carcass (chemical measurement). Based on DXA measurements, CLA did not result in an increase in either total body lean mass at 110 kg BW or carcass lean mass. In contrast, several studies reported that CLA resulted in an increase in lean mass or lean deposition [3, 5, 19, 20, 27]. There was no effect of CLA on bone deposition or bone density. In the study by Ostrowska et al. [20], DXA measurements of both the live animals and carcasses showed no effect of CLA on either bone growth or BMD. Other studies report no effect of dietary CLA supplementation on carcass ash content [19] or dissected bone weight [5].

#### 4.2. Interactions

The main objective of this study was to compare the response of control and IGF-I transgenic pigs to dietary CLA. Significant diet  $\times$  gene interactions were observed for the rate of fat deposition and total backfat deposition during growth from 60 to 110 kg BW. CLA (C-CLA) and the IGF-I transgene (TG-CO) resulted in the same level of reduction in the rate of fat deposition relative to control pigs (C-CO), however CLA did not lower the rate of fat deposition in TG pigs (TG-CLA). Backfat deposition was reduced by the IGF-I transgene but not by CLA, in fact, backfat deposition was greater in the TG-CLA pigs compared to TG-CO pigs. The reason for the observed greater increase in BF in the TG-CLA pigs compared to the TG-C pigs is not known and is not supported by the DXA measurements of gain in either the percentage or amount of fat gained during growth from 60 to 110 kg BW. The results of the DXA measurements showing that control pigs fed the CLA diet had less

fat and more lean than those fed the CO diet, are consistent with those of Ostrowska et al. [20]. Control pigs fed the CLA diet also had less carcass fat than those fed the CO diet, but only the results based on chemical analysis were statistically significant. Likewise, Ostrowska et al. [20] observed differences in chemical carcass composition, but not DXA carcass composition of control and CLA supplemented pigs. At 110 kg BW no differences in body composition were observed between TG-C and TG-CLA pigs, nor were there any differences in carcass composition between the two groups. These results indicate that the IGF-I transgenic pigs were less responsive to CLA than were their littermate controls. In the case of lean pigs, like the TG pigs in this study, it may be that CLA will not cause a further decrease in fat deposition, which is in line with previous reports [2, 8]. In contrast, CLA was reported to decrease backfat depth in lean contemporary genotypes housed under commercial conditions [6] and in stress-genotype pigs [29]. The ratio of fat-to-lean gain ranged from 0.40 for the TG-CLA pigs to 0.55 for C-CO pigs. For the control pigs in this study, the rates of fat deposition (267–314 g per day) and lean deposition (586–611 g per day) were comparable to those based on DXA measurements in the study reported by Ostrowska et al. [20] (249–334 and 467–556 g per day, respectively).

Various studies concerning the effects of CLA on the carcass composition of pigs have included measurements of the change in composition of specific body regions. With DXA total body scans, body region analysis is important because it allows the trunk region, that includes the viscera and other non-carcass components, to be analyzed separately from the limbs. In the present study, it was noted that both the live animal and carcass measurements by DXA indicated that the TG pigs less fat and more lean than control pigs in each region, whereas, the pigs fed the CLA diet had less fat in the shoulder and ham regions and more lean in each region compared to pigs

on the control diet. Dunshea et al. [6] noted that dietary CLA decreased the rate of accumulation of fat in the shoulder, particularly of gilts, resulting in a significantly lower amount of shoulder fat at slaughter. The study by Dugan et al. [5] reported less subcutaneous fat in all carcass regions (picnic, butt, loin and ham) of pigs that had been fed CLA. Swan et al. [26] measured a higher percentage of moisture and protein and a decreased percentage of lipids in the bellies of pigs fed a CLA-supplemented diet. A significant diet  $\times$  gene interaction for fat deposition in the trunk region indicates that the IGF-I transgene reduced fat deposition in that region, whereas there was a tendency for CLA to reduce fat deposition in trunk of control pigs, but to increase fat deposition in the TG pigs. Based on DXA live animal measurements, the TG pigs deposited more bone mineral in the trunk region. The DXA region measurements found no differences in bone content between pigs fed the CLA and control diets. Thiel-Cooper et al. [28] reported a dose dependent linear increase in dissected bone in the loin of pigs fed CLA, however, other studies report no effect of CLA on bone content of the loin [5] or the carcass [5, 19, 20].

In comparing the relative effects of CLA and the IGF-I transgene on the growth and carcass composition of pigs, the IGF-I transgene resulted in an increase in both growth rate and the efficiency of feed utilization, whereas CLA did neither. Both DXA and chemical analysis showed that the carcass of the TG-CO pigs contained less fat than that of the C-CLA pigs. The carcasses of TG pigs also had a higher percentage of lean (DXA) and a higher water content (chemical), however, there was no difference in bone (DXA), protein or ash (chemical) content. It has not been established at what point during growth, expression of the IGF-I transgene first becomes evident, however by 60 kg BW differences in body composition are significant as demonstrated in the present and in previous studies [21, 22]. Thus, the TG pigs already had an advantage in terms of improved body composition at

the time that the CLA feeding was started (60 kg BW). By comparing the response of the C-CLA pigs to the TG-CO pigs during the treatment period from 60 to 110 kg BW, it appears that the effects of CLA and the IGF-I transgene on the composition of growth were similar, with only a more rapid rate of lean deposition and less BF deposition occurring in the TG pigs compared to controls fed CLA.

#### **4.3. The use of DXA to measure body and carcass composition**

In addition to comparing the effects of CLA and the IGF-I transgene, this study demonstrates that one of the main benefits of DXA is the ability to evaluate the composition of growth of each individual within the timeframe of a specific treatment. Within the relatively narrow window of growth between 60 and 110 kg, the beginning composition of the pig can influence its final composition. Thus, the measurements of the composition of growth (Tab. III) are a better indicator of the effects of CLA than either the final live animal (Tab. II) or carcass (Tab. V) measurements. Validation studies have indicated that DXA is a potentially accurate and reliable method for estimating both live animal and carcass composition of swine [13, 17, 24, 26]. In the present study the comparison of DXA and chemical measurements of carcass composition confirm the accuracy of prediction equations for estimating the fat content of the carcass. The small but significant differences in estimation of lean and bone content suggest that additional refinement is needed for these measurements. The low correlation between DXA BMC and bone ash can be attributed in part to the difficulty in obtaining reliable ash values from ground carcass samples [17].

#### **5. CONCLUSIONS**

Overall, the effects of CLA were less than those of IGF-I transgene on body or



carcass composition; however, the main advantage of the IGF-transgene was realized prior to 60 kg BW. Thus, during the treatment period from 60 to 110 kg BW, the effects were similar, with only a more rapid rate of lean deposition and less BF deposition observed in the TG pigs compared to controls fed CLA. TG pigs did not respond to CLA as much as did control pigs, resulting in no difference in final body or carcass composition for TG pigs fed the CO or CLA diets.

In addition to the effects of the IGF-I transgene and CLA supplementation, the results of this study indicate that for final carcass evaluation, DXA was somewhat less sensitive than chemical measurements. However, the end point measurements provided by either DXA or chemical analysis were not as useful in assessing treatment response as were the comparative DXA "growth" measurements based on live total body scans at 60 and 110 kg BW.

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