RESEARCHREPORT

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PORK QUALITY

Tilte: Improved Meat Quality with Supplemental CLA – **NPB #98-143**

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Introduction. Selection of genotypes that provide a higher percentage of carcass lean has tended to produce carcasses with less desirable textural qualities. The fat is softer and the fat-muscle interface is often less cohesive. Lean pigs also have thin bellies, which presents two problems to the swine industry. First, thin soft bellies are difficult to process to bacon, and therefore, are sold at reduced value. Second, thin bellies produce thin bacon slices which may be unappealing to the consumer. The challenge to the industry it to produce lean pigs without compromising bacon quality. Conjugated linoleic acids have a variety of effects on the metabolism and growth of animals that may be exploited for improvement of pig production and pork quality.

Conjugated linoleic acids (CLA) are a group of geometric and positional isomers of linoleic acid. CLA are endproducts of bacterial metabolism of fatty acids and ruminant species absorb CLA from the digestive tract and incorporate these fatty acids in small amounts in milk and fat tissue. CLA isomers are also generated by heating linoleic acid in the presence of base. CLA has been linked to a multitude of metabolic actions, including reduced rates of fat accretion, increased saturation of fat, altered immune response, inhibition of carcinogenesis, and reduced serum lipids (Li and Watkins, 1998, Pariza et al. 2000). Of particular interest to animal scientist is the "nutrient partitioning" effect of CLA and the potential alteration of fatty acid profile. However, all of the listed responses are potentially beneficial to humans, so increasing the concentration of CLA in animal products has the added advantage of adding value to pork products. Therefore, interest in CLA extends beyond the potential benefits to the growth and composition of animals and into the role of animal products as a conveyor of therapeutic doses of CLA for human health.

Feeding CLA to rats dramatically reduces the rate of fat accretion and modestly increases the accretion of protein (Park et al., 1999). Work by several groups including our own suggests that the same beneficial effects occur in pigs (Heckart et al., 1999, Eggert et al., 1999). CLA may also effect the quality of pork. We and others have reported that CLA increases fat firmness and improves the quality of bacon and other products from lean pigs (Eggert et al., 1999). Numerous questions remain to be answered regarding the use of CLA including 1) are the growth and carcass quality

effects observed in lean genotype pigs, 2) what are the mechanisms by which CLA mediates such a wide variety of effects, 3) what isomers mediate specific metabolic effects, and 4) which isomers are beneficial to humans and what concentrations in animal tissues are beneficial. This work addressed the first two questions to determine the effect of genotype on the response to CLA, and to identify mechanisms for altered body composition.

Objectives

1. To determine if supplemental CLA increases the saturation of fatty acids in depot fat, improves the physical characteristics of depot fat, and improves meat and bacon quality in lean pigs.
2. To determine how CLA is altering fat metabolism by quantifying the gene expression or enzyme activity for key steps in fat synthesis.

Experimental design. Sixty early weaned gilts from two population obtained from commercial sources were segregated and housed in a curtain sided building at Purdue University swine unit. The two lines were a high-lean (HL) Large-While terminal sire x (large White x landrace) dam with an expected 54% fat free lean. The average genetics line (AL) was a Neu-Dalland terminal sire x Camborough cross with an expected 52% fat free lean. At 23 kg gilts were assigned to either 0.6% CLA or 1% sunflower oil in a standard corn soybean meal ration. Three pigs from each line were slaughtered at 46, 68, 91 114, and 136 kg. All management practices were approved by the Animal Care and Use committee.

Pigs were slaughtered at the Purdue meat lab and carcass and bacon assessed by trained personnel. Fresh pork color, firmness and marbling score at the cut surface of the 10^{th} and 11^{th} rib interface were evaluated at 2°C 24 h after exsanguination. Bellies were analyzed for firmness using a flop test, which suspends the belly over a horizontal bar. Standard measures of loineye area and backfat depth will be taken for estimation of composition. Samples of backfat and belly were taken for analysis of fatty acid composition by gas chromatography.

Adipose tissue collection and incubation. Subcutaneous adipose tissue from over the 10^{th} rib was removed within minutes of exsanguination. The tissue was cubed and either snap frozen in liquid nitrogen for mRNA analysis or placed in 37°C media consisting of M199, 25 mM Hepes and 0.5% BSA and returned to the laboratory. Tissue was sliced into 300 μm sections and small pieces (45 mg total) used for incubations. Tissue was placed into 3 mL incubation media containing Krebs Ringer Bicarbonate, 25 mM Hepes, 5mM glucose, 3% BSA and 2000mU/mL adenosine deaminase. Lipogenic rates were measured over 2h by the incorporation of U-[^{14}C]glucose in the presence of varying concentrations of insulin (0, 10 or 100 nM). Incubations were terminated by placing samples on ice. Explants were weighed and total lipid extracted. Radioactivity incorporated into total lipid was determined by scintillation counting. Adipocyte size was determined by fixing sliced adipose tissue in osmium tetroxide and determining cell diameter of fixed cells.

To determine the effect of CLA on expression of lipogenic genes RNA was extracted (Chomczynski and Sacchi, 1987) and used for hybridization to cDNA probes using both northern blots and ribonuclease protection assays.

Data were analyzed by the GLM procedure of SAS. Analysis of lipogenic data included insulin concentration, diet, genotype, slaughter weight, effects of pig nested within treatment and the appropriate interactions while the adipocyte size model included diet, genotype, weight, and interactions. Pig (genotype x diet x slaughter

weight) was the random term to test for main effects and interactions. When insulin was included, effects were tested using the residual error. Multiple comparisons were determined using the Duncan's procedure of the means statement in SAS. Single degree of freedom contrasts were used for preplanned comparison between individual treatment means.

Results

Animal Performance and carcass quality. HL pigs ate less and grew slower than AL pigs, but had a similar efficiency of gain (Table 1). The addition of CLA to the diet did not affect performance. Overall, HL pigs had less fat and greater fat-free lean than AL pigs (Table 1). CLA reduced total fat and increased fat-free lean in both genotypes. Responses were more apparent in the AL pigs.

Carcass quality measures were put on a three-point scale and data are recorded accordingly. HL pigs had less detectable marbling and color than AL pigs, but were similar for firmness and belly flop (Table 2). CLA was equally effective in both genotypes in improving carcass quality. Marbling, color, firmness and belly flop were all increased by CLA.

Adipose tissue metabolism. Work is in progress on aspects of adipose tissue metabolism and gene regulation, but preliminary data is presented. High lean pigs had smaller fat cells than AL pigs, reflecting reduced adipose tissue mass (Table 3). CLA reduced cell size in AL pigs but not HL pigs, suggesting that CLA was more effective in reducing fat accretion in AL pigs. The same pattern is shown for lipogenesis assessed in vitro. Across all insulin concentrations, fatty acid synthesis was lower in HL pigs fed the control diet (Table 4). Feeding CLA reduced lipogenic capacity 50% in AL pigs but only 17% in HL pigs. Insulin increased glucose incorporation into lipids 2 to 3 fold for each treatment group and CLA appeared to have no consistent effect on the response.

Treatment differences in lipogenic capacity suggest that CLA may affect the expression of key genes regulating lipogenesis. We have measured the mRNA abundance for fatty acid synthase and determined that mRNA abundance in HL pigs is only about 60% that of AL pigs. Decreased FAS is consistent with lower rates of fat accretion. Further, CLA reduced ($P<.05$) FAS expression in AL pigs, but actually increased expression in HL pigs. The same trend was present for expression of stearoyl CoA desaturase, the enzyme responsible for adding double bonds to newly synthesized stearic acid. Expression of the desaturase was lower in HL pigs and was decreased by CLA in AL but increased in HL pigs. Reduced desaturase activity would be expected to result in a fatty acid profile that contains more saturated fatty acids. These results suggest that CLA may increase in the saturation of fatty acids in some genotypes but not others.

Discussion

Lean pigs present a challenge to the swine industry because of reduced carcass quality. The physical properties of fat including firmness and cohesiveness are reduced, in part, because the fatty acid profile is more unsaturated and the percent water is increased (Wood 1984). Eating quality is not diminished but product presentation and the ability to process bacon and other cuts is compromised diminishing the value of the carcass. CLA has dramatic effects on growth and body composition in lab animals where rates of fat accretion are markedly reduced (Pariza et al., 2000). The response in pigs has been less consistent with little or no effect on

performance and a tendency for reduced fat accretion (Eggert et al, 1999 Sparks et al., 1999). More consistent has been the increased firmness of bellies in CLA-fed pigs (Eggert et al, 1999, Thiel et al., 1999). Further, CLA increases detectable marbling (Eggert et al, 1999). In the present study, CLA did not affect pig performance but increased the fat-free lean, marbling, and belly firmness. Results are consistent with previous reports and confirm the potential value of CLA to favorably shift the composition of gain and to improve the textural properties of fat and add value to pork products. Marbling scores were also improved with CLA indicating that alterations of fat metabolism are depot-specific and that energy may be channeled away from subcutaneous fat and toward intramusclular fat. Equally important is the finding that CLA effects were evident in both average-lean and high-lean pigs. The reduction in carcass fat was more evident in the AL line, but the improvement in marbling and belly firmness was similar in both lines. Therefore, the objective for feeding CLA may determine the economics of the addition. For instance, if carcass quality is not an issue, the benefit of feeding CLA to lean pigs may be small.

Genotype differences in response to CLA were further identified in adipose tissue metabolism. In Al pigs, CLA decreased the expression of FAS, the rate of lipogenesis and fat cell size. These responses are consistent with CLA interfering with fat accretion and likely account of the reduction in carcass fat. Curiously, CLA did not affect these parameters in HL pigs and carcass fat was less affected. The mechanism for CLA action is not well defined and likely involves multiple paths. To add to the complexity, CLA is a mixture of isomers which may have independent effects (Pariza et al., 2000). Most feed-grade sources of CLA contain near equal concentrations of cis-9,trans-11 and trans-10,cis-12 with smaller amounts of other isomers (Pariza et al., 2000). The trans-10,cis-12 is most closely associated with growth in mice (Pariza et al., 2000). The anti-diabetic properties of CLA appear to be mediated through the trans-10,cis-12 isomer as well (Ryder et al., 1999). The anti-diabetic action of CLA has may be mediated through activation of the transcription factor PPAR-δ (Belury et al., Ryder et al., 1999). However, it is unlikely that PPAR-δ mediates all of the effects of CLA, but exactly how CLA mediates the response is not clear.

CLA has been shown to decrease the expression of stearoyl-CoA desaturase mRNA in hepatocytes (Park et al., 1997) and adipocytes (Lee et al., 1998). In this study, CLA decreased the desaturase in AL pigs but not HL pigs. The rationale for measuring this gene is that reduced expression may account for increased fat firmness. A decrease with CLA feeding is consistent increased firmness in AL pigs, but firmness was also increased in HL pigs where the desarutase mRNA was not affected. Direct effects of CLA on desaturase activity have also been observed (Pariza et al., 2000), which may suggest that CLA effects both gene expression and enzyme activity.

Overall, CLA has several beneficial effects on pork production including reduced fat accretion in subcutaneous depots, but increased intramuscular fat, and increased firmness of bellies. Responses are evident in lean pigs but are less dramatic than in pigs with greater fat accretion. How CLA is mediating these effects and which isomers are responsible for the effects remain to be determined.

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Table 1. Effect of genotype and CLA on growth and carcass traits. Data are means for the entire study 50-300lbs

Variable	**High Lean**		**Average Lean**					
	CLA	SFO	CLA	SFO	SEM	G	D	G*D
ADG (lb./d)	1.97	1.92	2.04	2.04	.04	.02	.54	.63
F/G	3.09	2.98	3.01	3.05	.07	.92	.62	.35
FI (lbs./d)	5.78	5.52	5.81	5.99	.14	.08	.79	.13
Last rib (in)	1.1	1.13	.92	1.13	.04	.04	.003	.04
l. lumbar	.63	.64	.74	.94	.05	.0001	.03	.06
Outer	.25	.29	.27	.3	.01	.0001	.0006	.9
Middle	.22	.22	.28	.35	.02	.0001	.08	.11
Inner	.11	.13	.15	.18	.01	.0001	.06	.88
LEA	7.57	7.2	7.09	6.9	.2	.053	.166	.64
FFL	55.98	54.46	53.71	52.05	.67	.0008	.02	.92

Table 2. Effect of genotype and CLA on carcass quality

Variable	**High Lean**		**Average Lean**					
	CLA	SFO	CLA	SFO	SEM	G	D	G*D
Flop*	2.67	2.0	2.59	1.86	.1	.25	.0001	.78
Color	2.22	1.93	2.34	2.12	.07	.028	.0004	.60
Firmness	2.18	1.97	2.27	2.00	.07	.41	.001	.70
Marbling	1.79	1.51	2.14	1.75	.1	.005	.001	.6

* Flop (1=limp, 3= firm). Color (1=pale, 3=deep color), Frimness (1=soft, 3=very firm), marbling (1=low, 3=high)

Table 3. Effect of genotype and CLA on fat cell diameter.

Variable	**High Lean**		**Average Lean**					
	CLA	SFO	CLA	SFO	SEM	G	D	G*D
Diameter (um)	42.8	41.0	48.1	52.3		.01	.12	

Table 4. Effect of genotype and CLA on lipogenesis in adipose tissue.

Variable	**High Lean**		**Average Lean**					
	CLA	SFO	CLA	SFO	SEM	G	D	G*D
Lipogenesis*	0.95	1.15	0.84	2.37		.01	.12	
Insulin (0)	0.43	0.61	0.55	1.5				
Insulin (10)	1.18	1.36	0.97	2.53				
Insulin (100)	1.24	1.48	1.0	3.06				
Insulin response (fold increase)	2.9	2.4	1.8	2.0				

* rates are nmoles glucose incorporated/ 2h/ million cells