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Lipid Composition of Carcass Tissue from Transgenic Pigs Expressing a Bovine Growth Hormone Gene^{1,2}

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ABSTRACT: Fatty acid profiles and cholesterol content of whole-carcass ground tissue were compared from 26 transgenic (T) pigs expressing a bovine growth hormone gene (bGH) to 26 sibling control (C) pigs. All pigs were fed a common diet and were slaughtered at five different live weights: 14, 28, 48, 68, and 92 kg. The left side of each intact carcass was ground and tissue samples were analyzed for lipid composition and cholesterol content. At 14-kg body weight, carcasses from bGH-T pigs contained 38% less fat, 44% less saturated fatty acids (SFA), 48% less monounsaturated fatty acids (MUFA), and 38% less polyunsaturated fatty acids (PUFA) than C pigs. At 28 kg, bGH-T pigs had 38% less total carcass fat, 42%

less SFA, 46% less MUFA, and 24% less PUFA than C pigs. At 48-kg body weight, bGH-T pigs contained 48% less carcass fat, 55% less SFA, 59% less MUFA, and 22% less PUFA than C pigs. At 68 kg, bGH-T pigs had 78% less carcass fat, 78% less SFA, 79% less MUFA, and 53% less PUFA than C pigs. At 92 kg, carcasses from bGH-T pigs contained 85% less carcass fat, 85% less SFA, 91% less MUFA, and 66% less PUFA than those from C pigs. Cholesterol content was not different between bGH-T pigs and C pigs at any of the carcass weights. The trend was for cholesterol content to decrease from the 14- to 92-kg weight group. These results suggest a dilution effect of carcass fat and fatty acids in carcass tissue from bGH-T pigs with increasing live weight.

Key Words: Pigs, Transgenic, bGH, Carcass Fat, Fatty Acids, Cholesterol

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Introduction

The technology for introducing recombinant genes into mammals has been available only since 1980. At that time, five independent research groups succeeded in transferring genes to produce the first "transgenic" mice (see review by Palmiter and Brinster, 1986). Subsequently, constructs encoding growth-regulating genes, such as growth hormone (GH) or growth hormone-releasing factor (GHRF), were transferred to produce "super" mice (Palmiter et al., 1982). These results prompted the development of microinjection techniques for farm animals (Wall et al., 1985), which were then used to produce transgenic livestock (Ham-

mer et al., 1985). Most of the gene transfer experiments carried out with farm animals during the late 1980s attempted to manipulate growth and related production characteristics such as feed efficiency. By the end of the 1980s, the introduction of 16 genes had been reported for the three major livestock species (Pursel et al., 1989).

Gene transfer into farm animals is required to evaluate the potential for improving production efficiency, carcass quality, and disease resistance of livestock. It is well established that administration of exogenous porcine somatotropin (pST) to pigs at different stages of growth and development (e.g., between 25 and 110 kg) improves performance and results in alterations in body composition (Campbell et al., 1988, 1989; Smith and Kasson, 1990). However, little is known regarding body or carcass composition and lipid composition of transgenic farm animals that are expressing a GH transgene. Therefore, the purpose of this study was to determine what effect the expression of a recombinant bovine (b) GH gene in pigs has on lipid and carcass composition.

¹Mention of specific equipment and trade name does not imply endorsement by USDA.

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Materials and Methods

Animals and Feed. Fifty-two bGH transgenic and sibling control pigs were G2 generation descendants of transgenic founder 31-04 or G3 and G4 generation descendants of transgenic founder 37-06. The founders of these two bGH transgenic lines were produced by microinjection of fertilized ova with a metallothionein-I (MT)-bGH fusion gene (Pursel et al., 1989). Subsequent generations were produced by artificial insemination of nontransgenic crossbred females with fresh or frozen epididymal spermatozoa recovered from bGH transgenic boars after euthanasia. Presence of the bGH transgene was established by hybridization of dot blots of DNA from tail biopsies (Hammer et al., 1985). Expression of the bGH transgene was established by radioimmunoassay of plasma collected at 1 wk of age or older using a bGH radioimmunoassay that did not measure pGH (Miller et al., 1989). Control pigs were siblings that did not contain the transgene. Plasma bGH concentrations were > 1,000 ng/mL in transgenic pigs of the 31-04 line and approximately 60 ng/mL in transgenic pigs of the 37-06 line (Pursel et al., 1989). Carcass composition was not affected by the different lines of transgenic founder. Pigs were weaned at 4 to 8 wk of age and had ad libitum access to a corn-soybean meal diet (Table 1) containing 18% crude protein, supplemented with .25% lysine (3.8 Mcal of DE/kg). Pigs were processed at five different live weights: 14, 28, 48, 68, and 92 kg. This research was approved by our institutional animal care committee.

Experimental. The left side of each intact carcass (skin and hair included) was ground in a whole-carcass grinder and random samples of ground tissue were collected. Duplicate tissue samples (10 g) were analyzed for lipid composition and tissue cholesterol content.

Isolation of Lipids. An Omni-Mixer homogenizer and the procedure of Folch et al. (1957) were employed to obtain lipid extracts of each sample. Tissue samples were homogenized three times in a 2:1 chloroform:methanol mixture (vol/vol). A .58% aqueous NaCl solution was added to the homogenate, causing the chloroform layer (containing lipid) to separate from the methanol-water phase. In the extract, chloroform, methanol, and water (.58% NaCl) plus water contributed from the tissue were in the proportions 8:4:3 by volume. Aliquots of the lipid extract were transferred to screw-cap test tubes (16 mm × 125 mm) for subsequent cholesterol and fatty acid analysis.

Fatty Acid Analysis. Fatty acids were converted into methyl ester derivatives before GLC analysis. Methyl henicosanoate (C21:0) (3.96 mg/mL) served as the internal standard. Along with each set of samples esterified, a triglyceride standard (tripalmitin, 3.44 mg/mL) and control sample were included to confirm the consistency and accuracy of the results. The

Table 1. Composition of diet^a

Ingredient	Percentage of total (as fed)
Corn	63.3
Soybean meal	16.0
Dried skim milk	12.0
Lysine hydrochloride	.25
Corn oil	4.0
Dicalcium phosphate	3.0
Iodized salt	.5
Selenium premix	.05
Mineral premix ^b	.3
Vitamin premix ^c	.3

^aCalculated nutrient composition: CP, 18%; total lysine, 1.2%.

^bProvided the following micronutrients in parts per million per kilogram of complete diet: Mn, 150; Fe, 150; Cu, 15; Co, 1.5; I, 4.5; Zn, 150.

^cProvided the following per kilogram of complete diet: vitamin A, 6,600 IU; vitamin D, 1,200 IU; riboflavin, 13.2 mg; pantothenic acid, 26.4 mg; niacin, 46.2 mg; vitamin B₁₂, 44 µg; choline, 330 mg; vitamin E, 16.5 IU.

control sample was a lipid extract from a randomly selected loin sample analyzed in the study. Methylation and preparation of samples for GLC analysis were previously described (Solomon et al., 1990).

Analysis of the fatty acid composition was performed on a gas chromatograph (Model 5880A, Hewlett-Packard, Avondale, PA) designed to accommodate a capillary column. The gas chromatographic technique of Slover and Lanza (1979) was followed to quantify fatty acids resolved on a 30-m SP 2330 fused silica capillary column (Supelco, Bellefonte, PA). Helium served as the carrier and makeup gas (column flow rate = .8 mL/min and makeup flow rate = 30 mL/min). Temperatures of the injector, oven, and detector were 200, 170, and 200°C, respectively.

Cholesterol Analysis. Cholesterol was converted to a trimethylsilyl ether derivative; stigmaterol (.40 mg/mL) served as the internal standard. The derivatizing procedure and sample preparation were previously described (Solomon et al., 1990).

Analysis of the cholesterol was performed on a gas chromatograph (Hewlett-Packard) designed to accommodate a packed column. A 183-cm × 4-mm glass column (Alltech Associates, Deerfield, IL) packed with 3% OV-17 on Gas Chrom Q (100/120) was used to resolve the cholesterol and stigmaterol peaks. Helium served as the carrier gas; column flow rate was = 50 mL/min. Temperatures of the injector, oven, and detector were 300, 270, and 300°C, respectively.

Data Analysis. Data were analyzed by the least squares analysis of variance using the GLM routine (SAS, 1985) to determine the significance of variation between transgenic and controls at the five different weight groups for a 2 × 5 factorial arrangement, which included treatment × weight group interactions. The comparison of weight groups was based on linear and quadratic orthogonal polynomials.

Table 2. Comparison of total carcass lipid, cholesterol content, and fatty acid profiles for transgenic and control pigs

Item	Weight group, kg										SEM
	Controls (C)					Transgenics (bGH-T)					
	14	28	48	68	92	14	28	48	68	92	
N	4	4	5	5	8	4	4	5	5	8	
Total lipid, g/100 g ^a	10.04	12.32	16.58	26.78	29.07	6.19	7.62	8.16	5.97	4.49	.67
Cholesterol, mg/100 g ^a	100.88	94.97	85.92	74.15	77.81	106.45	99.97	86.28	74.55	77.08	1.20
Fatty acids, g/100 ^a											
C14:0	.15	.14	.19	.30	.27	.08	.07	.08	.05	.03	.01
C16:0	2.32	2.46	3.49	5.07	4.74	1.43	1.45	1.58	1.05	.76	.16
C16:1- <i>trans</i>	.05	.05	.04	.04	.04	.03	.03	.04	.03	.02	0
C16:1- <i>cis</i>	.56	.37	.44	.47	.43	.30	.13	.13	.06	.03	.03
C17:0	.05	.03	.04	.10	.05	.03	.02	.03	.03	.02	0
C18:0	.72	1.04	1.64	2.72	2.58	.39	.67	.70	.48	.35	.07
C18:1	3.09	4.20	6.16	8.08	8.11	1.64	2.26	2.30	1.45	1.06	.26
C18:2	1.38	2.25	2.72	3.81	3.76	1.01	1.75	1.93	1.58	1.26	.18
C18:3	.04	.05	.06	.08	.08	.02	.01	.03	.02	.01	0
C20:0	0 ^b	.01	.01	.04	.03	0	0	0	0	0	0
C20:1	.04	.07	.10	.15	.15	.02	.04	.04	.03	.02	.01
C20:4	.08	.08	.09	.07	.08	.05	.06	.06	.03	.02	.01
Total saturated fatty acids	.324	3.69	5.37	8.22	7.67	1.92	2.20	2.39	1.60	1.15	.23
Monounsaturated fatty acids	3.74	4.69	6.73	8.73	8.73	1.98	2.47	2.50	1.57	1.13	.29
Polyunsaturated fatty acids	1.51	2.38	2.87	3.95	3.91	1.08	1.82	2.02	1.63	1.29	.19

^aWet weight basis.^b0 = <.01 on nondetectable.

Results and Discussion

Feed intake was not recorded for these pigs; however, from previous work with similar transgenic pigs (Pursel et al., 1989) a 20% reduction in feed intake could be expected for transgenic pigs. Average daily gains (not in tabular form) were not significantly different between control and transgenic pigs in the present experiment. Backfat thickness and longissimus muscle area (LMA) were measured (not in tabular form) at the 10th rib for only the pigs in the 48-, 68-, and 92-kg weight groups. Backfat increased from 16.0 mm to 22.4 mm for the control (C) pigs. Conversely, backfat decreased from 8.1 to 2.8 mm for the bGH-transgenic (T) pigs. The LMA increased in both the control and bGH-T pigs with increasing body weight and no difference at any of the weight groups was detected between bGH-T and sibling C pigs.

Carcass fat deposition followed a different pattern for bGH-T pigs and C pigs (Table 2). Total carcass lipid increased linearly (190%) in the C pigs from 14- to 92-kg live weight, whereas for the bGH-T pigs total carcass lipid only increased (32%) from 14- to 48-kg live weight and then decreased (45%) from 48 to 92 kg (overall 27% decrease from 14 to 92 kg).

At 14-kg body weight, carcasses from bGH-T pigs contained 38% less fat than those from C pigs. At 28 kg, bGH-T pigs had 38% less total carcass fat; at 48

kg, 48% less total carcass fat; at 68 kg, 78% less total carcass fat; and at 92 kg, 85% less carcass fat than C pigs. In fact, some of the 92-kg bGH-T pigs contained less than 3% total carcass fat. Studies involving the effect of daily pST administration to pigs reported decreases in carcass fat, but not of this magnitude (Campbell et al., 1990; Caperna et al., 1990). Total cholesterol content of the ground carcass tissue was not different between bGH-T and C pigs at any of the designated weights; however, with increasing live weight (14 to 92 kg), cholesterol content decreased for both C and bGH-T pigs (23% C pigs; 28% bGH-T pigs). Carcass weight had both a negative linear and a positive quadratic effect on cholesterol content of the carcass. Similar results for muscle and adipose tissue were found in pigs receiving daily administration of pST (Solomon, 1992). Although the role of dietary cholesterol intake in humans is questionable, the general recommendation is to limit intake to 100 mg/1,000 kcal, not to exceed 300 mg/d (American Heart Association, 1986).

Carcasses from bGH-T pigs consistently contained less ($P < .01$) total saturated fatty acids (SFA) than those from C pigs at each slaughter weight (Table 2). A 137% linear increase in total SFA was observed in C pigs with increasing live weight (14 to 92 kg), compared with a 40% decrease in total SFA for bGH-T pigs. These differences in SFA were mostly a result of changes in C16:0 (palmitic acid), C18:0 (stearic

Table 2 (continued).

Treatment	Significance, <i>P</i> <				Treatment × weight group
	Weight group				
	Linear		Quadratic		
	bGH-T	C	bGH-T	C	
.01	.18	.01	.12	.81	.01
NS	.01	.01	.01	.01	NS
.01	.05	.01	.64	.45	.01
.01	.07	.01	.35	.18	.01
.01	.19	.57	.29	.29	.39
.01	.01	.45	.32	.40	.60
.01	.43	.01	.12	.05	.01
.01	.41	.01	.05	.01	.01
.01	.18	.01	.19	.04	.01
.01	.90	.01	.06	.17	.01
.01	.68	.01	.42	.53	.05
.01	1.0	.01	1.0	.16	.01
.01	.89	.01	.25	.29	.01
.01	.01	.31	.06	.93	.14
.01	.10	.01	.20	.08	.01
.01	.12	.01	.26	.08	.01
.01	.97	.01	.06	.17	.01

acid), and C14:0 (myristic acid). Margaric (C17:0) and arachidic (C20:0) acids were also present, but in very small quantities. The bGH-T pigs from all weight groups were devoid of C20:0, whereas the only C pigs that did not contain C20:0 were the 14-kg pigs.

Palmitic acid accounted for 62% of the total detectable SFA in the C pigs (92 kg weight group), compared with 66% in bGH-T pigs. Stearic acid accounted for 34% of the total SFA in the C pigs (92-kg weight group), compared with 30% in bGH-T pigs. A 104% increase was observed for palmitic acid in C pigs with increasing live weight from 14 to 92 kg, compared with a 47% reduction in bGH-T pigs. A 258% linear increase was observed for stearic acid in C pigs with increasing live weight, compared with a 10% reduction in bGH-T pigs. Myristic acid increased by 80% in C pigs, compared with a 63% decrease in bGH-T pigs with increasing live weight. The administration of exogenous pST to growing pigs resulted in lean tissue containing 40% less SFA and subcutaneous fat containing 33% less SFA than controls (Solomon, 1992). The majority of this difference was attributed to less palmitic and stearic acids in the carcass tissue from pST pigs. Clark et al. (1992) reported similar results regarding SFA content of lean and subcutaneous fat of pST-treated pigs.

Both myristic acid and palmitic acid have been reported to be hyperlipidemic and hypercholesterolemic (Keys et al., 1965). Human consumption of hypercholesterolemic fatty acids has come under attack by health professionals (NRC, 1988). Bona-

mone and Grundy (1988) demonstrated that a diet high in stearic acid did not elevate plasma levels of low-density lipoprotein cholesterol, perhaps because it is poorly digested and can be easily desaturated to oleic acid (Keys et al., 1965). At 14-kg body weight, carcasses from bGH-T pigs contained 44% less SFA than those from C pigs. At 28 kg, bGH-T pigs had 42% less SFA; at 48 kg, bGH-T pigs had 55% less SFA; at 68 kg, bGH-T pigs had 78% less SFA; and at 92 kg, bGH-T pigs contained 85% less SFA than did C pigs. From a dietary viewpoint, carcass tissue from bGH-T pigs reflects a much more favorable (lower) quantity of SFA.

Carcasses from bGH-T pigs contained less total monounsaturated fatty acids (MUFA) than did those from C pigs at each processing weight (Table 2). The fatty acids that constituted the MUFA were palmitoleic (C16:1-*cis*), palmitelaidic (C16:1-*trans*), oleic (C18:1), and *cis*-11-eicosenoic (C20:1) acid. Oleic acid was the most abundant MUFA in the total lipid fraction and more *cis*-C16:1 was present. The bGH-T pigs contained 87% less oleic acid than C pigs (92 kg live weight). The trend was for the quantity of MUFA to increase with increasing body weight in C pigs. A MUFA dilution effect was observed in bGH-T pigs after 48-kg live weight that was the same pattern for total carcass lipid deposition. At 14-kg body weight, carcasses from bGH-T pigs contained 48% less MUFA; at 28 kg, 46% less MUFA; at 48 kg, 59% less MUFA; at 68 kg, 79% less MUFA, and at 92 kg, 91% less MUFA than those from C pigs. Solomon (1992) found a 37% reduction in lean tissue MUFA and a 24% reduction in subcutaneous fat MUFA when exogenous pST was administered daily to growing pigs. Clark et al. (1992) reported similar results regarding the MUFA content of lean and subcutaneous fat of pST-treated pigs. Oleic acid has been reported to be hypolipidemic, reducing both cholesterol and LDL triglycerides (Grundy, 1986), and for that reason it is not considered to be an undesirable dietary fatty acid. Mattson and Grundy (1985) demonstrated that oleic acid had the ability to reduce low-density lipoprotein cholesterol. Although C pigs contained a higher proportion of oleic acid, a lower fatty acid content, regardless of the type of fatty acid, would still be more desirable.

Significant differences in the percentage of polyunsaturated fatty acids (PUFA) were observed in the carcass tissue from C and T pigs (Table 2). Carcass tissue from bGH-T pigs (92-kg live weight) contained 66% less C18:2 (linoleic acid), 88% less C18:3 (linolenic acid), and 75% less C20:4 (arachidonic acid), for an overall 67% reduction in total PUFA, than did that from C pigs. Polyunsaturated fatty acids have received much less criticism with regard to dietary detriments; in fact, they have been reported to be hypolipidemic/hypocholesterolemic (NRC, 1988). At 14-kg slaughter weight, carcasses from bGH-T pigs

contained 38% less PUFA; at 28 kg, 24% less PUFA; at 48 kg, 22% less PUFA; at 68 kg, 53% less PUFA, and at 92 kg, 66% less PUFA than those from C pigs. The trend was for PUFA to increase with increasing live weight in C pigs and to decrease with increasing weight in bGH-T pigs. Administration of exogenous pST had a minor effect on PUFA content of muscle or adipose tissue of pigs (Clark et al., 1992; Solomon, 1992).

Implications

Carcass tissue from bovine growth hormone transgenic pigs reflects much more favorable levels of fatty acids than does that from control pigs when considering dietary health recommendations and concerns. If levels of growth hormone secretion could be precisely regulated during the rapid growth phase of market hogs, it might be possible to provide consumers with an ultra-low-fat pork product.

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