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Growth performance, carcass characteristics, meat quality, and tissue histology of growing pigs fed crude glycerin-supplemented diets[1]

P. J. Lammers,* B. J. Kerr,†[2] T. E. Weber,† K. Bregendahl,* S. M. Lonergan,* K. J. Prusa,‡ D. U. Ahn,* W. C. Stoffregen,§ W. A. Dozier III,# and M. S. Honeyman*

*Department of Animal Science, Iowa State University, Ames 50011; †USDA-ARS, Swine Odor and Manure Management Research Unit, Ames, IA 50011; ‡Department of Food Science and Human Nutrition, Iowa State University, Ames 50011; §USDA-ARS, Bacterial Diseases of Livestock Research Unit, Ames, IA 50011; and #USDA-ARS, Poultry Research Unit, Mississippi State, MS 39762

ABSTRACT: The effects of dietary crude glycerin on growth performance, carcass characteristics, meat quality indices, and tissue histology in growing pigs were determined in a 138-d feeding trial. Crude glycerin utilized in the trial contained 84.51% glycerin, 11.95% water, 2.91% sodium chloride, and 0.32% methanol. Eight days postweaning, 96 pigs (48 barrows and 48 gilts, average BW of 7.9 ± 0.4 kg) were allotted to 24 pens (4 pigs/pen), with sex and BW balanced at the start of the experiment. Dietary treatments were 0, 5, and 10% crude glycerin inclusion in corn-soybean meal-based diets and were randomly assigned to pens. Diets were offered ad libitum in meal form and formulated to be equal in ME, sodium, chloride, and Lys, with other AA balanced on an ideal AA basis. Pigs and feeders were weighed every other week to determine ADG, ADFI, and G:F. At the end of the trial, all pigs were scanned using real-time ultrasound and subsequently slaughtered at a commercial abattoir. Blood samples were collected pretransport and at the time of slaughter for plasma metabolite analysis. In addition, kidney, liver, and eye tissues were collected for subsequent examination for lesions characteristic of methanol toxicity. After an overnight chilling of the carcass, loins were removed for meat quality, sensory evaluation, and fatty acid profile analysis. Pig growth, feed intake, and G:F were not affected by dietary treatment. Dietary treatment did not affect 10th-rib backfat, LM area, percent fat free lean, meat quality, or sensory evaluation. Loin ultimate pH was increased ($P = 0.06$) in pigs fed the 5 and 10% crude glycerin compared with pigs fed no crude glycerin (5.65 and 5.65 versus 5.57, respectively). Fatty acid profile of the LM was slightly changed by diet with the LM from pigs fed 10% crude glycerin having less linoleic acid ($P < 0.01$) and more eicosapentaenoic acid ($P = 0.02$) than pigs fed the 0 or 5% crude glycerin diets. Dietary treatment did not affect blood metabolites or frequency of lesions in the examined tissues. This experiment demonstrated that pigs can be fed up to 10% crude glycerin with no effect on pig performance, carcass composition, meat quality, or lesion scores.

Key words: biofuel, crude glycerin, fatty acid, growing pig, histology, meat quality

INTRODUCTION

The production of biofuels is increasing in a dramatic fashion (Ma and Hanna, 1999; Hill et al., 2006; Kurki et al., 2006). Biodiesel production in the United States has grown exponentially since 1999 with existing US production capacity being approximately 5.3 billion L annually (National Biodiesel Board, 2007). A co-product of the biodiesel industry is crude glycerin, with approximately 79 g of crude glycerin generated for every 1.0 L of biodiesel produced (Thompson and He, 2006). Consequently, with current biodiesel production capacity, approximately 4.2×10^8 kg of crude glycerin could be generated annually (National Biodiesel Board, 2007).

Crude glycerin obtained from a biodiesel production facility using soybean oil as its feedstock has been shown to be a highly available energy source in laying hens (Lammers et al., 2008a), broilers (Dozier et al., 2008), and swine (Lammers et al., 2008b). Pigs can be fed up to 10% glycerin with little effect on pig performance (Kijora et al., 1995, 1997). The reported effect of glycerin on meat quality, however, has been inconsistent. In pigs fed wheat-soybean meal-based diets, 24-h drip loss and cooking loss were reduced in the muscles from pigs supplemented with 5% crude glycerin (Mourot et al., 1994). In contrast, Kijora and Kupsch (2006) noted no effect on carcass dripping or press water loss in pigs fed barley-soybean meal-based diets supplemented with up to 10% glycerin. Crude glycerin supplementation has been shown to slightly increase oleic acid at the expense of linoleic and linolenic acids, consequently decreasing the unsaturation index of fat (Mourot et al., 1994; Kijora et al., 1997).

Low concentrations of methanol are contained in crude glycerin and acute methanol intoxication can lead to formic acid accumulation leading to metabolic acidosis (Medinsky and Dorman, 1995; Skrzydlewska, 2003). Animals differ widely, however, in their ability to metabolize methanol (Roe, 1982). Although crude glycerin contains trace concentrations of methanol, no data exist on the effect of crude glycerin containing methanol on tissue histology in growing pigs. The objectives of the current study were to examine the effects of crude glycerin supplementation on growing-finishing pig performance, carcass composition, meat quality, composition and profile of LM intramuscular lipid, and histology of the eye, liver, and kidney tissues.

MATERIALS AND METHODS

The Iowa State University Animal Care and Use Committee approved all experimental protocols.

Animals and Dietary Treatments

Crude glycerin was obtained from a biodiesel production facility (AG Processing Inc., Sergeant Bluff, IA) that utilized soybean oil as its feedstock. The analysis of the crude glycerin used in this study is listed in Table 1.

Pigs (Cambrough 22 females × L337 sires) were weaned at 21 d of age and fed a commercial starter diet for 1 wk. Eight days postweaning, 96 pigs (48 gilts and 48 barrows) with an average BW of 7.9 ± 0.4 kg were allotted to 24 pens (4 pigs/pen) with sex distribution and pen weight balanced at the start of the experiment. Dietary treatments were randomly assigned to pens, with dietary treatments being 0, 5, and 10% crude glycerin inclusion in corn-soybean meal diets. Pigs were fed diets over a 5-phase feeding program during the 138-d trial. Within each phase, diets were offered ad libitum in meal form and were formulated to be equal in ME, sodium, chloride, with diets based on total Lys and other AA balanced on an ideal AA basis (NRC, 1998). Initial diet formulation and calculated nutrient content of control diets are summarized in Table 2.

Pigs were individually weighed every other week with feed disappearance recorded at the time of pig weighing to determine ADG, ADFI, and G:F. Dietary phase changes corresponded with the day that pigs were weighed, occurring on the same day for all treatments. Pigs were housed in nursery (1.2 × 1.2 m) pens for 33 d, grower (1.8 × 1.9 m) pens for 28 d, and finisher (2.7 × 1.8 m) pens for the final 77 d. Nursery pens had wire mesh flooring, and the grower and finisher pens had partial slats. All rooms were mechanically ventilated with pull-plug manure storage systems. During the course of the experiment, 6 pigs were removed due to health issues with no pattern of pig removal related to dietary treatment and no individual pen having more than 1 pig removed. Pen feed disappearance was adjusted for the removed pig at the time of removal. On d 138, all pigs were weighed (133 ± 6 kg of BW) for the termination of the performance period and scanned using real-time ultrasound as described by Sullivan et al. (2007). In addition, blood samples (10 mL) for plasma analysis were collected via jugular venipuncture into containers containing sodium heparin. In addition, each pig was bled via jugular venipuncture into heparinized tubes (14.3 USP units/mL) and stored on ice until blood collection from all pigs was complete. Samples were then centrifuged at $900 \times g$ for 20 min at 4ºC, after which an aliquot of plasma from each sample was used for plasma urea nitrogen analysis. Pigs remained in their respective pens with access to their respective diets and water until transport to the abattoir on d 139.

Carcass Traits

On the morning of d 139, ninety pigs were transported approximately 300 km to the abattoir (Sioux-Preme Packing Co., Sioux Center, IA). One pig died during transport. On d 140, pigs were electrically stunned and exsanguinated. Blood, eye, and liver samples were harvested from early postmortem carcasses for further analysis. Carcasses were chilled overnight (0°C). Last rib fat depth was measured on each carcass at 24 h postmortem, and the percent lean was calculated (proprietary equation, Sioux-Preme Packing Co.). The loin from the left side of each carcass (10th rib to posterior tip) was removed, vacuum packaged, placed on ice, transported to Iowa State University, and stored at 0°C until subsequent analysis. Tissue and loin samples from 2 pigs were not collected at the abattoir due to operator error. Loin marbling scores were evaluated 12 d postmortem according to National Pork Board Standards (NPPC, 2000). Loin muscle was evaluated for moisture composition (AOAC, 1995) with loin purge determined on additional loin samples after 12 d of storage using methodology described by Gardner et al. (2006). Following loin purge loss, chop purge was

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Table 1. Characterization of crude glycerin

Item	Value	Analytical method
Total glycerin,[1] %	84.51	Determined by difference[2]
Methanol,[1] %	0.32	Gas chromatography (proprietary method)
pH[1]	5.67	Orion 230A pH meter with 9107 BN probe
Total fatty acids,[1] %	0.00	AOCS[3] G 4.40 modified for glycerin
Moisture,[4] %	11.95	AOAC[5] 984.20
CP,[4] %	0.82	AOAC 990.03
Crude fat,[4] %	0.23	AOAC 920.39 (A)
Ash,[4] %	2.98	AOAC 942.05
Sodium,[4] %	1.20	AOAC 956.01
Chloride,[4] %	1.71	AOAC 9.15.01, 943.01
Potassium,[4] %	<0.005	AOAC 956.01
Color[4]	<1	AOCS Cc 13a-43
ME,[6] kcal/kg	3,638	Predicted value

[1]Values reported by AGP Inc., Sergeant Bluff, IA (Lot # GB608–25).

[2]Determined within the AGP Inc. laboratory as: $100 - \%$ methanol $- \%$ total fatty acid $- \%$ moisture $- \%$ NaCl.

[3]AOCS (2000).

[4]Analysis by University of Missouri-Columbia Experiment Station Chemical Laboratories, Columbia, MO.

[5]AOAC (1995).

[6]ME of crude glycerin = GE of pure glycerin × purity of crude glycerin = 4,305 kcal/kg × 84.51% (Lammers et al., 2008b).

Table 2. Initial diet formulation and calculated nutrient content of control experimental diets fed to pigs[1]

Item	Phase 1 (7 to 12 kg of BW)	Phase 2 (12 to 23 kg of BW)	Phase 3 (23 to 45 kg of BW)	Phase 4 (45 to 78 kg of BW)	Phase 5 (78 to 120 kg of BW)
Ingredient, %					
Corn	54.00	64.50	69.50	77.30	84.40
Soybean meal	30.90	30.90	26.50	20.00	13.50
Soybean oil	1.00	0.65	0.65	—	—
Dried whey	10.00	—	—	—	—
L-Lysine·HCl	0.27	0.17	0.15	0.10	0.09
DL-Methionine	0.09	0.06	—	—	—
L-Threonine	0.09	0.07	0.03	—	—
Dicalcium phosphate	2.12	2.05	1.42	1.10	0.76
Ground limestone	0.73	0.70	1.00	0.77	0.55
Sodium chloride	0.26	0.40	0.35	0.33	0.30
Choline chloride, 60%	0.03	—	—	—	—
Vitamin premix[2,3]	0.35	0.35	0.25	0.25	0.25
Mineral premix[4,5]	0.16	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
ME, kcal/kg	3,294	3,305	3,327	3,319	3,343
Lysine, %	1.37	1.23	1.10	0.88	0.70
TSAA, %	0.76	0.73	0.61	0.53	0.47
Threonine, %	0.89	0.83	0.72	0.58	0.49
Tryptophan, %	0.25	0.24	0.21	0.18	0.14
Calcium, %	0.93	0.83	0.80	0.62	0.45
Available phosphorus, %	0.54	0.46	0.34	0.27	0.20
Sodium, %	0.21	0.18	0.16	0.15	0.14
Chlorine, %	0.34	0.29	0.26	0.25	0.23

[1]Dietary treatments 5 and 10 consisted of the above diets formulated to include 5 or 10% crude glycerin, respectively, whereas calculated ME, listed amino acid content, available phosphorus, sodium, and chlorine remained constant. Crude glycerin replaced 7 to 10% corn and 40 to 60% sodium chloride at a 5% level and 15 to 17% corn and 80 to 100% sodium chloride at a 10% level.

[2]Provided the following per kilogram in phase 1 and 2 diets: vitamin A, 7,718 IU; vitamin E, 40 IU; niacin, 57 mg; D-pantothenic acid, 31 mg; riboflavin, 12 mg.

[3]Provided the following per kilogram in phase 3 through 5 diets: vitamin A, 5,513 IU; vitamin E, 29 IU; niacin, 42 mg; D-pantothenic acid, 22 mg; riboflavin, 8 mg.

[4]Provided the following per kilogram in phase 1 diet: Zn, 156 mg as ZnO; Fe, 280 mg as Fe_2SO_4; Cu, 1.4 mg as CuO; Mn, 73 mg as MnO_2; I, 3.2 mg as CaI.

[5]Provided the following per kilogram of phase 2 through 5 diets: Zn, 146 mg as ZnO; Fe, 263 mg as Fe_2SO_4; Cu, 1.3 mg as CuO; Mn, 68 mg as MnO_2; I, 3.0 mg as CaI.

determined using 2.54-cm-thick chops, which were weighed and placed in a plastic bag and stored for 24 h at 2.2 ± 1.1°C, with chop purge based on the weight of free liquid in the bag (Gardner et al., 2006). Drip loss was determined using 2.54-cm-thick boneless chops (2 per loin) as described by Lonergan et al. (2001). Minolta color values from each chop were obtained with a Minolta Chroma meter (model CR-310; Konica Minolta Sensing Americas Inc., Ramsey, NY) with a 0° viewing angle, a 50-mm diameter measuring area, and a CIE D_{65} illuminant. One measurement was taken on each chop sample.

Cooked Loin Evaluation

The loins of 2 pigs from each pen (1 barrow and 1 gilt) were randomly selected for evaluation. Following 12 d of storage, two 2.54-cm-thick loin chops were removed from the center of the loin for sensory and instrumental texture analysis as described by Sullivan et al. (2007).

Fatty Acid Profile Analysis

Lipids were extracted and measured from a sample of each LM (Folch et al., 1957), which were subsequently methylated to fatty acid methyl esters using boron trifluoride (BF_3) in methanol, and removed from solution as described by Du et al. (1999). The fatty acid methyl esters were analyzed for fatty acid composition according to procedures established by Du et al. (1999) using gas chromatography (HP 6890 equipped with an autosampler, flame ionization detector; Agilent Technologies, Santa Clara, CA) and a column (HP-wax fused silica capillary column, 30 m × 0.25 mm × 0.25 µm film thickness; Supelco, Bellefonte, PA). Fatty acid methyl esters were identified by comparing the retention times of fatty acid standards.

Plasma Metabolites

In addition to obtaining blood samples from all pigs before shipping, blood samples were also collected on the day of slaughter at the time of exsanguination into 50-mL centrifuge tubes containing sodium heparin (14.3 USP units/mL). Samples were subsequently centrifuged at 900 × g for 20 min at 4°C and stored at −80°C pending analysis. Plasma urea nitrogen was determined enzymatically as described previously (Kerr et al., 2004). Plasma cortisol was determined using a commercially available kit (DSL-10–2000, Active Cortisol EIA; Diagnostic Systems Laboratories Inc., Webster, TX) that has been previously validated for porcine serum (Weber and Spurlock, 2004). In addition, commercially available kits (Sigma Chemical Co., St. Louis, MO) were used to measure plasma glucose and glycerol (GAHK20 and F6428, respectively), and kits (Pointe Scientific Inc., Canton, MI) to measure plasma lactate concentrations and creatine kinase activity

(L7596 and C7512, respectively). All of the plasma metabolites were measured in duplicate.

Tissue Histology

From all pigs, 1 eye, liver, and kidney per pig were collected at the time of slaughter and placed in neutral-buffered 10% formalin. They were subsequently processed by routine paraffin embedding techniques, cut in 4-µm sections, and stained with hematoxylin and eosin and Masson's trichrome techniques. All sections were read for lesions (Maxie, 2007) twice by a single person versed in lesion evaluation.

Statistical Analysis

Data were subjected to ANOVA (SAS Inst. Inc., Cary, NC), and differences between means were tested using the PDIFF option. Pig performance (ADG, ADFI, and G:F) was evaluated in each dietary phase and for the entire 138-d feeding period with the pen used as the experimental unit. Carcass composition and meat quality traits were evaluated to test for effects of dietary treatment, pig sex, and diet × sex interaction, whereas plasma metabolites pretransport and immediately postexsanguination, and differences in the frequency of histological lesions were evaluated for the effect of dietary treatment. Individual pigs were the experimental unit for analysis of carcass composition, meat quality, plasma metabolites, and lesion data.

RESULTS AND DISCUSSION

Average daily gain, ADFI, and G:F were not affected by dietary treatment in any phase (data not shown) or over the entire 138-d period (Table 3). These results agree with results from previous studies examining growth performance of pigs fed crude glycerin in barley-soybean meal (Kijora et al., 1995, 1997; Kijora and Kupsch, 2006) and wheat-soybean meal diets (Mourot et al., 1994). This is also supported by work in broilers that demonstrated up to 5% glycerin can be fed without affecting growth or feed conversion (Simon et al., 1996; Cerrate et al., 2006).

The effects of diet, sex, and their potential interaction on carcass characteristics are described in Table 4. There was no diet × sex interaction on any trait examined. In agreement with other reports (Mourot et al., 1994; Kijora et al., 1995, 1997; Kijora and Kupsch, 2006), dietary treatment did not affect 10th-rib backfat, LM area, fat-free lean, daily lean gain, or carcass lean percentage. As expected, 10th-rib backfat was greater in barrows than gilts (Cline and Richert, 2001; Renaudeau and Mourot, 2007).

Diet did not affect HCW, percent loin lean, moisture content, or chop lipid percentage (Table 5). These results agree with other reports (Mourot et al., 1994; Kijora et al., 1995, 1997; Kijora and Kupsch, 2006). Inclu-

Table 3. Growth performance of growing pigs fed crude glycerin[1]

Item	Diet[2] 0	5	10	SEM	*P*-value
Replicates, pen[3]	8	8	8		
Start BW, kg	7.9	8.0	7.8	0.2	0.60
End BW, kg	132.9	134.0	132.8	2.3	0.92
ADG, g	905	913	906	16	0.93
ADFI, g	2,333	2,385	2,400	52	0.66
G:F, g/g	0.39	0.38	0.38	0.01	0.12

[1]Over the 138-d feeding trial.

[2]Dietary treatments were 0, 5, or 10% crude glycerin inclusion in corn-soybean meal diets fed in 5 phases.

[3]Four pigs were initially assigned to each pen; over the course of the experiment, 6 pigs were removed, with no pen having more than 1 pig removed.

sion of glycerin in the diet did not affect chop drip loss, which is in agreement with Kijora and Kupsch (2006) and Airhart et al. (2002), but contrary to the findings of Mourot et al. (1994). As expected, carcasses from gilts weighed less and were leaner than carcasses from barrows ($P \leq 0.05$).

Loin tissue from pigs fed 10% crude glycerin had less concentrations of linoleic acid (18:2) than the other dietary treatments ($P < 0.01$; Table 5), which agrees with the work of Mourot et al. (1994) and Kijora et al. (1997). Eicosapentaenoic acid (20:5) increased with increasing crude glycerin supplementation ($P = 0.02$). Mourot et al. (1994) did not report eicosapentaenoic acid (20:5) concentrations but reported declines in myristic acid (14:0) in backfat and linolenic acid (18:3) in backfat and semimembranosus muscle when pigs were fed 5% glycerin. Kijora et al. (1997) did not find these changes in backfat from pigs fed 10% glycerin. There is no clear consensus on the effect feeding crude glycerin may have on fatty acid profile of pork lipid (Mourot et al., 1994; Kijora et al., 1997). The apparent disagreement on the effect feeding glycerin has on fatty acid profile of pork fat may be due to differences in amount and profile of fatty acids remaining in the crude glycerin, or in our case, the reduction in corn (and consequently corn oil) due to the addition of crude glycerin. The relative

differences in amount and profile of fatty acids in other feedstuffs included in the experimental diets may also limit comparisons across studies.

Dietary glycerin may reduce water loss from the carcass and cooking if slaughter follows an overnight fast (Mourot et al., 1994). In the current experiment, however, pork loin quality and sensory characteristics were not affected by diet or sex and there was no diet × sex interaction (Table 6). Furthermore, data presented here indicate that cooking loss is not affected by crude glycerin supplementation, which is in contrast with the findings of Mourot et al. (1994) who reported less carcass drip loss and cooking loss from muscle of pigs fed 5% glycerin. The lack of a change in drip and cooking loss in the current study may be due to the 30-h feed withdrawal time compared with the overnight fast in the study by (Mourot et al., 1994). Other workers have demonstrated that removing feed 24 h before slaughter will reduce drip loss and lessens decline in muscle pH (Jones et al., 1985; Eikelenboom et al., 1990). Eikelenboom et al. (1990) also reported reduced cooking loss in pigs fasted 24 h before slaughter. This is the first report of sensory evaluation of loin chops from pigs fed crude glycerin.

There was no diet × time interaction or diet effect on any plasma metabolite measured (Table 7). Plasma urea

Table 4. Effect of crude glycerol on carcass characteristics[1]

Item	Diet[2] 0	5	10	SEM	Sex Barrow	Gilt	SEM	*P*-value Diet	Sex	D × S[3]
No. of pigs	30	29	31		44	46				
Initial BW, kg	8.0	8.0	7.9	0.2	7.9	8.0	0.2	0.80	0.78	0.69
Final BW, kg	133	134	133	2.0	137	129	2.0	0.93	0.01	0.92
10th-rib backfat, mm	18.8	21.0	20.7	0.8	22.0	18.3	0.7	0.14	0.01	0.13
LM area, cm[2]	48.6	49.0	46.6	0.9	48.0	48.1	0.7	0.12	0.92	0.33
Fat free lean, %	52.0	51.8	50.6	0.8	51.9	51.1	0.6	0.37	0.34	0.78
Lean gain, g/d	365	363	355	5.0	364	358	4.0	0.37	0.30	0.70
Carcass lean, %	55.7	54.7	55.7	0.5	55.5	55.2	0.4	0.26	0.48	0.32

[1]Based on ultrasound scan data except for percent carcass lean, which was obtained from measured last rib backfat using a lean matrix equation (propriety equation; Sioux-Preme Packing Co., Sioux Center, IA).

[2]Dietary treatments were 0, 5, or 10% crude glycerol inclusion in corn-soybean meal diets fed in 5 phases over a 138-d feeding trial.

[3]D × S: diet × sex interaction.

Table 5. Carcass characteristics and fatty acid profile of LM chop lipid from pigs fed crude glycerin

Item	Diet[1]			SEM	Sex		SEM	P-value		
	0	5	10		Barrow	Gilt		Diet	Sex	D × S[2]
No. of loins,	27	29	31		43	44				
HCW, kg	95.2	97.2	97.3	1.8	98.7	94.5	1.4	0.61	0.03	0.97
Loin lean, %	55.8	54.7	55.5	0.5	54.7	56.0	0.4	0.21	0.02	0.61
Loin moisture, %	74.0	73.9	74.0	0.1	73.8	74.1	0.1	0.78	0.01	0.78
Loin ultimate pH	5.57	5.65	5.65	0.03	5.63	5.62	0.02	0.06	0.77	0.59
Loin purge, %	1.67	1.84	1.62	0.17	1.77	1.65	0.13	0.61	0.54	0.43
Chop purge, %	3.72	3.84	3.90	0.30	3.70	3.94	0.20	0.90	0.46	0.24
Chop drip loss, %	0.85	0.73	0.81	0.10	0.79	0.80	0.08	0.67	0.96	0.87
Chop lipid, %	2.15	2.07	2.08	0.07	2.19	2.02	0.06	0.71	0.04	0.70
Fatty acid[3]										
14:0	1.29	1.31	1.25	0.03	1.30	1.27	0.02	0.06	0.03	0.04
16:0	24.10	24.14	24.15	0.19	24.29	23.97	0.16	0.98	0.15	0.48
16:1n-7	3.73	3.87	3.82	0.08	3.79	3.83	0.07	0.45	0.65	0.29
17:0	0.28	0.29	0.25	0.02	0.28	0.27	0.01	0.28	0.64	0.68
17:1n-10	0.27	0.30	0.30	0.01	0.29	0.29	0.01	0.08	0.68	0.59
18:0	11.68	11.77	12.00	0.18	11.86	11.78	0.14	0.41	0.69	0.39
18:1	39.47	38.92	40.18	0.44	39.90	39.14	0.36	0.12	0.13	0.75
18:2n-6	10.34	10.34	9.27	0.26	9.68	10.28	0.21	0.01	0.04	0.63
18:3n-3	0.27	0.29	0.27	0.02	0.26	0.30	0.02	0.78	0.17	0.65
20:0	0.13	0.13	0.13	0.01	0.13	0.13	0.01	0.55	0.82	0.75
20:4n-6	2.96	3.00	2.90	0.10	2.78	3.13	0.08	0.76	0.01	0.76
20:5n-3	0.09	0.10	0.11	0.01	0.09	0.10	0.01	0.02	0.05	0.90
22:5n-6	0.28	0.29	0.28	0.01	0.26	0.30	0.01	0.08	0.01	0.75

[1]Dietary treatments were 0, 5, or 10% crude glycerin inclusion in corn-soybean meal diets fed in 5 phases over a 138-d feeding trial. Loins were evaluated 12 d postmortem.

[2]D × S: diet × sex interaction.

[3]Fatty acids are expressed as g/100 g of total fatty acids. Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. The position of the first double bond relative to the methyl (n) end of the molecule is also included.

nitrogen is an indicator of body protein status (Kohn et al., 2005) and has been used to determine protein requirements and lean tissue growth rates in pigs (Chen et al., 1995; Coma et al., 1995). Plasma urea nitrogen was not affected by time of collection or diet, supporting the conclusion that lean tissue mobilization was not

Table 6. Meat quality and sensory evaluation of LM chops from pigs fed crude glycerin

Item	Diet[1]			SEM	Sex		SEM	P-value		
	0	5	10		Barrow	Gilt		Diet	Sex	D × S[2]
No. of chops	16	16	16		24	24				
Marbling score[3]	2.0	2.1	2.1	0.1	2.1	2.0	0.1	0.81	0.60	0.61
Cooking loss, %	18.3	17.9	18.6	0.9	18.7	17.9	0.7	0.86	0.45	0.79
Japanese color score[4]	2.6	2.7	2.8	0.8	2.7	2.7	0.1	0.79	0.83	0.51
Minolta L*[5]	55.6	55.3	55.6	0.8	55.8	55.1	0.7	0.95	0.48	0.42
Minolta a*[5]	17.5	17.4	17.4	0.2	17.3	17.6	0.1	0.88	0.23	0.09
Minolta b*[5]	4.9	5.1	4.6	0.4	4.9	4.9	0.3	0.68	0.94	0.12
Instron,[6] kg of force	6.0	5.9	6.0	0.3	6.2	5.7	0.2	0.91	0.10	0.14
Juiciness score[7]	5.5	5.7	5.5	0.4	5.4	5.7	0.3	0.93	0.54	0.35
Tenderness score[7]	6.1	6.1	5.9	0.4	5.8	6.3	0.3	0.93	0.24	0.29
Chewiness score[7]	3.6	3.4	3.3	0.3	3.5	3.3	0.2	0.74	0.39	0.31
Pork flavor score[7]	2.2	2.2	2.2	0.1	2.2	2.2	0.1	0.91	0.56	0.05
Off-flavor score[7]	3.5	3.4	3.1	0.3	3.2	3.5	0.3	0.68	0.35	0.23

[1]Dietary treatments were 0, 5, or 10% crude glycerin inclusion in corn-soybean meal diets fed in 5 phases over a 138-d feeding trial.

[2]D × S: diet × sex interaction.

[3]Evaluated 12 d postmortem according to National Pork Board Standards (NPPC, 2000). The marbling standards correspond to percentage of intramuscular lipid.

[4]Japanese color bar 1 to 6 scale, 1 = extremely light, 6 = extremely dark (Sullivan et al., 2007).

[5]Higher L* values indicate a lighter color, higher a* values indicate a redder color, and higher b* values indicate a more yellow color (Sullivan et al., 2007).

[6]Average of 3 maximum force peaks.

[7]Scores on a 1 to 10 scale. Lower scores represent low degrees of characteristics, high scores represent high degrees of characteristics (Sullivan et al., 2007).

Table 7. Effect of crude glycerin[1] on plasma metabolites pretransport and at the time of slaughter

Item	Pretransport[2]			Slaughter[2]			SEM	P-value		
	0	5	10	0	5	10		Diet	Time	D × T[3]
No. of pigs	30	29	31	29	29	31				
PUN,[4] mg/dL	14.7	14.5	13.6	14.0	14.6	13.8	0.5	0.24	0.67	0.59
Cortisol, µg/dL	6.7	6.6	6.1	15.1	11.8	13.6	1.6	0.56	0.01	0.59
Glucose, mg/dL	101.8	99.0	98.0	138.6	143.4	140.3	4.6	0.91	0.01	0.70
Glycerol, µM	0.04	0.04	0.04	417.5	410.3	444.8	34.7	0.87	0.01	0.87
Lactate, mM	4.0	4.7	4.1	12.4	12.3	12.2	0.6	0.86	0.01	0.83
CPK,[5] IU/L	720.2	683.3	678.0	1,844.2	2,212.7	1,954.8	110.3	0.29	0.01	0.19

[1]Dietary treatments were 0, 5, or 10% crude glycerin inclusion in corn-soybean meal diets fed in 5 phases over the 138-d feeding trial.

[2]Blood samples for plasma analysis were collected before transport to the abattoir and at the time of slaughter immediately after electrical stunning.

[3]D × T = diet × time interaction.

[4]PUN = plasma urea nitrogen.

[5]CPK = creatine phosphokinase.

altered by feeding up to 10% crude glycerin. Glycerin is absorbed by the gastrointestinal tract of nonruminants (Tao et al., 1983) and crude glycerin has been shown to be a source of energy in both pigs (Lammers et al., 2008b) and chickens (Dozier et al., 2008; Lammers et al., 2008a). The absence of a dietary treatment effect on plasma glycerol concentrations indicates metabolism of dietary glycerin was not affected at values less than or equal to 10% of the diet. Concentrations of most plasma metabolites were different between pretransport and at the time of slaughter ($P < 0.01$). Transporting pigs has been shown to cause stress in pigs (Pérez et al., 2002; Apple et al., 2005). Increases in plasma cortisol, glucose, lactate, and creatine phosphokinase are correlated with increased stress in pigs (Brown et al., 1998; Pérez et al., 2002; Apple et al., 2005). Our results indicate a stress response in pigs following transport to the abattoir, and that feeding crude glycerin did not reduce this effect.

Current biodiesel processing techniques utilize methanol, which is not completely recovered, and thus, methanol is found in crude glycerin at very low concentrations (Table 1). Intermediates in the metabolism of methanol to carbon dioxide and water are formalde-hyde and formate. The toxic effects due to methanol poisoning are actually due to the formation, accumulation, and slow metabolism of formate in some species (Medinsky and Dorman, 1995; Skrzydlewska, 2003). Clinical consequences of methanol poisoning are central nervous system depression, vomiting, severe metabolic acidosis, blindness, and Parkinsonian-like motor disease (Roe, 1982; Dorman et al., 1993; Soffritti et al., 2002; Skrzydlewska, 2003). During the course of this study, no pig demonstrated any clinical signs of methanol toxicity. The 6 animals that were removed during the trial were removed for respiratory disease or lameness, with no attribution to a specific dietary treatment. Of the 89 pigs slaughtered, no gross lesions were observed at the time of collection. In addition, the frequency of histological lesions in kidney, liver, and eye, the pharmacological targets for methanol toxicity, were not influenced by dietary treatment (Table 8). This agrees with an earlier study in which no pathological changes were observed in liver or kidney in response to consumption of crude glycerin during finishing (Kijora et al., 1995).

Provided diets are formulated on an equal energy basis, the results from this study demonstrate that up to

Table 8. Frequency of histological lesions in tissue of pigs fed crude glycerin, % of tissues with lesion[1]

Lesion	Diet[2]			SEM	P-value
	0	5	10		
Hepatocellular pleomorphism	93.1	96.6	96.8	4.0	0.75
Portal hepatitis	41.3	34.5	45.1	9.2	0.70
Periportal fibrosis	27.6	17.2	12.9	7.3	0.34
Lymphoplasmacytic interstitial nephritis	41.4	41.4	48.4	9.4	0.82
Lymphoplasmacytic hepatitis	3.4	3.4	3.2	3.4	0.99
Lymphohistiocytic perineuritis	0.0	3.4	0.0	2.0	0.36
Hepatic lipidosis	3.4	0.0	0.0	2.0	0.35

[1]No gross lesions were observed in tissues harvested. One eye, liver, and kidney were collected from 29, 29, and 31 pigs for diets 0, 5, and 10, respectively.

[2]Dietary treatments were 0, 5, or 10% crude glycerin inclusion in corn-soybean meal diets fed in 5 phases over a 138-d feeding trial.

10% crude glycerin can be fed to growing-finishing pigs with little or no effect on growth performance, carcass composition, meat quality, or lesion scores in the eye, liver, or kidney tissue. Although we noted only small effects on ultimate pH and fatty acid profiles of the LM, the decline in drip and cooking losses as reported by (Mourot et al., 1994) may warrant further examination of the effect of crude glycerin supplementation on meat quality through evaluation of the amount, method, or length of administration. Combined with our previous work evaluating the energy value of crude glycerin in nonruminants (Dozier et al., 2008; Lammers et al., 2008a,b), we conclude that crude glycerin is a viable source of dietary energy that is well utilized by pigs. Lastly, although this study was not designed to specifically examine the toxicology of methanol fed to pigs, the results indicate that the concentrations of methanol in these diets did not negatively affect pig performance or frequency of histological lesions in tissues associated with methanol metabolism.

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