CSIRO PUBLISHING

Animal Production Science, 2009, **49**, 154–161 www.publish.csiro.au/journals/an

A chemical analysis of samples of crude glycerol from the production of biodiesel in Australia, and the effects of feeding crude glycerol to growing-finishing pigs on performance, plasma metabolites and meat quality at slaughter

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Abstract. The aims of this study were to: (i) determine the chemical composition of 11 samples of crude glycerol collected from seven Australian biodiesel manufacturers; and (ii) examine the effects of increasing levels of crude glycerol fed to growing-finishing pigs on performance, plasma metabolites and meat quality at slaughter. Chemical composition of crude glycerol samples varied considerably; glycerol content ranged between 38 and 96%, with some samples containing up to 29% ash and 14% methanol. One of these samples (76.1% glycerol, 1.83% methanol) was then fed to 64 female pigs (50.9 ± 5.55 kg; mean $\pm$ s.d.) allocated to one of five dietary treatments (0, 4, 8, 12 and 16% crude glycerol) until they reached 105 kg liveweight. There were no statistical differences in performance indices with increasing levels of added glycerol, although there was an unexpectedly high variation between treatments. Blood glycerol levels were unaffected by diet in week two of the experiment, but increased linearly ($P < 0.001$) with increasing levels of dietary glycerol before slaughter. The inclusion of crude glycerol did not influence any meat quality parameters at slaughter ($P > 0.05$). Diets containing added crude glycerol were less dusty after mixing, but diets that contained 8, 12 and 16% glycerol all formed a firm aggregate within 24 h of mixing that presented some feeding difficulties. This might restrict inclusion of glycerol in mash diets to dietary levels less than 8%. Furthermore, levels of residues such as methanol and ash should be monitored to prevent excessive amounts of these compounds in pig diets.

Introduction

Biodiesel is an alternative automotive fuel that can be produced from vegetable oils and (or) animal fats. In general, the oil or fat is mixed with an alcohol, usually methanol, and a catalyst (often sodium hydroxide) that causes triglycerides to separate, forming methyl esters (biodiesel) and crude glycerol. Crude glycerol is the principal co-product of biodiesel production (Ma and Hanna 1999; Gerpen 2005; Thompson and He 2006) and has been proposed as a potential beneficial energy source for pigs (Lammers *et al*. 2008*a*) and poultry (Dozier *et al*. 2008; Lammers *et al*. 2008*b*). Key factors, however, determining the nutritional value of crude glycerol for pigs are the feedstock source and the manufacturing process (Thompson and He 2006).

Studies in numerous species have demonstrated equivocal results with respect to improvements in production with added glycerol (see review by Kerr *et al*. 2007), although there are reports that dietary inclusion of glycerol can improve meat quality. Mourot *et al*. (1994), for example, reported reduced drip and cooking losses in pigs fed 5% added glycerol. The quality of the crude glycerol product from biodiesel manufacture and its suitability for inclusion in pig diets in Australia are currently unknown, as are its likely effects on performance and metabolism.

The aims of the present study were 2-fold: (i) test the chemical composition of samples of crude glycerol obtained from biodiesel manufacturers in Australia, and (ii) investigate the effects of feeding crude glycerol in diets for pigs on performance, the concentrations of selected plasma metabolites, and indices of meat quality at slaughter.

Materials and methods

This study was approved by the Animal Ethics Committees at Murdoch University and the Western Australian Department of Agriculture and Food to ensure compliance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

Analysis of crude glycerol samples

Samples of crude glycerol were donated from different biodiesel factories in Australia for subsequent determination of pH, density

 10.1071/EA08210 1836-0939/09/020154

(ISO 12185), glycerol (ISO 2879-1975), moisture (ISO 12937), ash (ISO 6245), methanol (GC-FID) and matter organic non-glycerol (MONG), defined as 100 – (glycerol content, % + water content, % + ash content, %) (ISO 2464). All analyses were conducted by ASG-Analytic Pty Ltd, Largs Bay, SA, Australia or at ASG Analytik-Service GmBH, Neusäss, Germany.

Animals, housing and diets

Sixty-four Large White × Landrace female pigs weighing 50.9 ± 5.55 kg (mean ± s.d.) were used in an unbalanced, randomised block study to examine the effects of added crude glycerol (0, 4, 8, 12 and 16%) in the diet until market weight at 105.2 ± 3.85 kg (mean ± s.d.) liveweight. Pigs were housed individually in pens measuring 1.8 × 0.9 m on a fully slatted concrete floor in a naturally ventilated shed. Each pen had a single-space feeder and a nipple water drinker. The 64 pigs were allocated to 12 blocks according to weight and then randomly allocated to one of the five dietary treatments. Sixteen pigs were allocated to the control diet without crude glycerol and 12 pigs to each of the four diets with added crude glycerol. The pigs had *ad libitum* access to feed and water throughout the study except for the days before bleeding where the pigs were fasted overnight.

The five dietary treatments were mash-based diets as follows: (1) control diet; (2) diet with 4% added crude glycerol; (3) diet with 8% added crude glycerol; (4) diet with 12% added crude glycerol; and (5) diet with 16% added crude glycerol. Diets were formulated to meet or exceed the nutrient requirements for pigs of this genotype. Crude glycerol was added to the diet at the expense of wheat and barley, such that all of the diets formulated contained the same energy and total amino acid contents (Table 1). The crude glycerol used in the present feeding trial was produced from a mix of vegetable oil and tallow, contained 76.1% glycerol and an unexpectedly high methanol content of 1.83% (batch I in Table 2). The crude glycerol was estimated to have an approximate digestible energy (DE) value of 13.0 MJ/kg, based on the work of Lammers *et al*. (2008*a*) who found that crude glycerol with a glycerol content of 87% contained 14.0 MJ DE/kg.

Measurements and sampling

Measurements made on pigs were the production indices (feed intake, daily weight gain, feed conversion ratio and P2 back fat), circulating levels of metabolites in plasma (glycerol, glucose and free fatty acids), and indices of meat quality (pH, drip loss, cook loss, colour and shear force).

Liveweight and feed intake of individual pigs were recorded on a weekly basis. The pigs were bled 2 weeks after the start of the experiment and ~1 week before expected slaughter via jugular

Table 1. Composition and analyses of the experimental diets (as-fed basis)

Diet no.:	1	2	3	4	5
Crude glycerol addition (%):	0	4	8	12	16
Ingredient (g/kg)					
Barley	191.6	167.1	142.6	118.0	100
Wheat	592.4	577.3	562.1	547.0	514.9
Lupin	100	100	100	100	100
Soybean meal, 48% crude protein	40	40	40	40	51.1
Blood meal	20	20	20	20	20
Meat meal	30	30	30	30	30
Crude glycerol	–	40	80	120	160
Limestone	9.83	9.02	8.20	7.39	6.54
Dicalcium phosphate	11.5	11.7	11.9	12.0	12.2
NaCl	2	2	2	2	2
L-lysine	1.06	1.21	1.36	1.52	1.33
DL-methionine	0.41	0.46	0.52	0.57	0.57
L-threonine	0.05	0.16	0.28	0.39	0.36
Mineral/mineral premix[A]	0.70	0.70	0.70	0.70	0.70
Choline chloride	0.40	0.40	0.40	0.40	0.40
Calculated content					
Digestible energy (MJ/kg)	13.5	13.5	13.5	13.5	13.5
Crude fat (g/kg)	20.7	21.3	21.2	21.1	21.0
Crude fibre (g/kg)	36.9	35.7	34.2	33.1	31.9
Crude protein (g/kg)	164.0	162.6	160.0	160.0	160.0
Total lysine (g/kg)	8.59	8.60	8.58	8.59	8.59
Analysed content					
Gross energy (MJ/kg)	16.52	16.58	16.61	16.64	16.61
Crude protein (g/kg)	146.3	146.3	149.1	149.1	149.1
Glycerol (g/kg)	<5.0	43.0	75.0	108.0	130.0
Methanol (g/kg)	0.1	0.2	0.2	0.2	0.2

[A]Supplied per kg of diet: 60.0 mg Fe ($FeSO_4$); 10.0 mg Cu ($CuSO_4$); 40.0 mg Mn (MnO); 100.0 mg Zn (ZnO); 0.30 mg Se (Na_2SeO_3); 0.50 mg I (KI); 0.20 Co ($CoSO_4$); vitamin A, 7000 IU; vitamin D_3, 1400 IU; vitamin E, 20.0 mg; vitamin K_3, 1.0 mg; thiamin, 1.0 mg; riboflavin, 3.0 mg; pyridoxine, 1.5 mg; vitamin B_{12}, 0.015 mg; pantothenic acid, 10.0 mg; folic acid, 0.2 mg; niacin, 12.0 mg and biotin 0.03 mg.

Table 2. Characteristics of crude glycerol from different biodiesel plants in Australia

Batch[A]	Content of parameters characterised						
	pH	Density (g/cm^3)	Glycerol (%)	Moisture (%)	Ash (%)	Methanol (%)	MONG[B] (%)
A	3.3	1.25	77.0	16.1	2.3	<0.01	4.6
B[1]	5.4	1.25	94.8	2.0	0.0	<0.01	3.2
C[1]	7.6	1.21	96.5	1.3	0.0	<0.01	1.0
D	9.0	1.07	38.4	0.3	4.2	0.14	57.0
E	2.3	1.20	61.1	2.5	29.4	0.23	6.8
F[2]	10.6	1.13	66.7	0.2	2.9	11.40	18.8
G[2]	10.8	1.12	64.5	0.0	3.4	13.94	18.1
H[3]	2.7	1.25	83.4	10.7	1.5	0.18	4.2
I[3]	2.0	1.26	76.1	11.7	3.5	1.83	6.9
J[3]	2.4	1.25	74.5	14.3	4.6	0.55	6.7
K	8.6	1.19	63.4	1.0	5.6	4.72	25.3
Average	5.9	1.20	72.4	5.5	5.2	4.12	13.9
Min.	2.0	1.07	38.4	0.0	0.0	<0.01	1.0
Max.	10.8	1.26	96.5	16.1	29.4	13.94	57.0

[A]Batches with the same superscript originated from the same plant.
[B]MONG: matter organic non-glycerol. Defined as 100 – [glycerol content (%) + water content (%) + ash content (%)].

vein puncture into 10-mL lithium-heparin-coated vacutainer tubes. The blood samples were centrifuged at 2000*g* for 10 min at 5°C to recover plasma that was stored at −20°C until analysed for glycerol, glucose and non-esterified fatty acid (NEFA) contents. Pigs were fasted overnight for ~12 h before blood sampling occurred.

Plasma concentrations of glycerol, glucose and NEFAs were determined by an enzymatic colourimetric method using glycerol kinase (Roche Diagnostics, Indianapolis, IN), hexokinase (Roche Diagnostics) and acyl-CoA synthetase (WAKO NEFA-C Kit, Novachem Pty Ltd, Collingwood, Vic., Australia), respectively.

Dietary gross energy (GE) content was determined using a ballistic bomb calorimeter (SANYO Gallenkamp, Loughborough, Leics, UK) and dietary nitrogen (N) content was determined by combustion analysis (LECO FP-428) (Association of Official Analytical Chemists official method 990.03) (AOAC 1997). Crude protein was calculated by multiplying the N content by 6.25. The contents of glycerol (ISO 2879-1975) and methanol (GC-FID) in crude samples and diets were determined by ASG Analytik-Service in either Largs Bay or Neusäss. Identical methods were used in both laboratories.

Slaughter procedures and meat quality assessments

The pigs were slaughtered at a commercial abattoir over a 5-week period when they reached a liveweight of ~105 kg. The pigs were transported for ~1.5 h to the abattoir where they had a lairage period of ~1.5 h before undergoing carbon dioxide anaesthesia and subsequent exsanguination. Carcass weight and depth of back fat at the P2 site (6.5 cm from the midline over the last rib) were measured on the slaughter line using a Hennessy Grading Probe 4 (Hennessy Grading Systems Limited, Auckland, New Zealand).

At 45 min after exsanguination, the pH of the *M. longissimus dorsi* (*l. dorsi*) was measured using a portable pH/temperature meter (Jenco Electronic Ltd, Sydney, NSW, model 6009), fitted with a polypropylene spear-type gel electrode (Ionode IJ42S, Brisbane, Qld, Australia) and a temperature probe. Twenty-four hours after slaughter, a portion of the *l. dorsi* was collected from the left side of the chilled carcass and measurements of objective pork quality carried out. The pH of the *l. dorsi* was measured in the same manner as above and sections of *l. dorsi* were standardised to 20 mm in thickness and allowed to bloom for 10 min before determination of colour and pH. The pH was measured as described whereas surface colour was determined as lightness, redness and yellowness with a Minolta Chromameter CR-400 (Konica Minolta Sensing, Inc., Tokyo, Japan) using D65 lighting, a 2-degree standard observer and 8-mm aperture in the measuring head standardised to a white tile. Drip loss was measured in duplicates using the suspension method (Honikel 1998) where a rectangular cube of muscle from the *l. dorsi* (avoiding fat deposits) was cut to an approximate weight of 20 g ± 1 g. The sample was placed in plastic netting and suspended in an inflated and sealed plastic bag for a further 24 h at 5°C. Samples were then removed from the bag, gently blotted dry with paper towel and reweighed.

Meat samples for determination of cook loss and shear force were prepared and cooked as described by Bouton *et al*. (1971) with the modifications mentioned by Channon *et al*. (2003). Quadratic blocks of the *l. dorsi* without external fat and epimysium were cut to an approximate weight of 100 g ± 2 g, placed in individual plastic bags, suspended in a water bath and cooked for 1 h at 80°C. Thereafter, the bags were cooled for 30 min in cold running water after which the samples were removed from the plastic bags, gently patted dry with a paper towel and reweighed. The samples were then placed in new plastic bags and refrigerated overnight at 5°C. The next day the refrigerated cooked sample was cut into five subsamples of 1.5 cm wide, parallel to the muscle fibres, and 0.7 cm thick for determination of tenderness expressed as shear force. Objective tenderness was measured using a Warner-Bratzler shear force

blade fitted to an Instron Universal testing machine (High Wycombe, UK, Model 1122; Tension load cell type 2511-104 Model A30-40 UK 1233) with a crosshead speed of 200 mm/min. A 10-kN load cell measured the peak force required to shear muscle fibres.

Statistical analysis

All statistical analyses were performed using SAS (version 9.0; SAS Institute Inc., Cary, NC). Performance traits were analysed univariately in normal linear models using the SAS GLM procedure, with starting weight used as a covariable and block and dietary glycerol level included in the model. The effects of glycerol inclusion levels on plasma metabolites and meat quality indices were analysed according to the same normal linear model, but without starting weight as a covariable. Polynomial regression was used to determine the presence of linear or quadratic treatment effects as crude glycerol levels were increased. Data on weekly feed intake consistency were analysed as repeated-measures using a Gaussian model of spatial correlation in the MIXED procedure of SAS. The individual pig served as the experimental unit in the analyses. All means are reported as least- squares means. Treatment effects were considered significant at $P < 0.05$, whereas $P < 0.10$ was considered a trend. In order to ensure homogeneity of variance it was necessary to logarithmically transform the plasma concentration of NEFAs. The back-transformed values and confidence intervals are presented herein.

Results

Crude glycerol analyses

The colour of the crude glycerol samples ranged from light brown, almost clear, to very dark brown, nearly black. All but one sample (batch D) was liquid at room temperature. Chemical characterisation of the crude glycerol samples is given in Table 2. There was a large variation in the chemical content of the different samples, and some samples contained significant ash and methanol residues.

Diets

The analysed GE content of the diets was similar between diets, whereas the analysed level of crude protein was lower than expected, but again, similar across diets (Table 1). The actual glycerol content in the diets was close to the planned levels when correcting for the added crude glycerol containing 76% pure glycerol. It is noteworthy that the analysed methanol levels in the diets were around detection levels despite the crude glycerol added containing 1.83% methanol.

Using a liquid crude glycerol as in the present experiment presented diet mixing and feed-flow problems, even though including crude glycerol in the diet reduced feed dust. In particular, the diets containing 12 and 16% crude glycerol left the vertical auger in the mixer with a sticky coating, and the diets containing 8, 12 and 16% crude glycerol all formed a firm aggregate within 24 h after mixing.

Production indices

Average daily feed intake and average daily gain of the pigs decreased linearly with an increasing amount of crude glycerol in the diet in the first week of the study (Fig. 1); however, this was reversed by the end of the second week as pigs adapted to the diets (Fig. 2). Overall, average daily feed intake was lower ($P = 0.026$) for the pigs fed the diet containing 8% crude glycerol compared with the pigs fed the diets containing 4 and 12% crude glycerol mainly due to a lower feed intake in the last period of the trial. In contrast, overall daily weight gain, feed conversion ratio and P2 back fat were unaffected ($P > 0.05$) by up to 16% crude glycerol in the diet (Table 3).

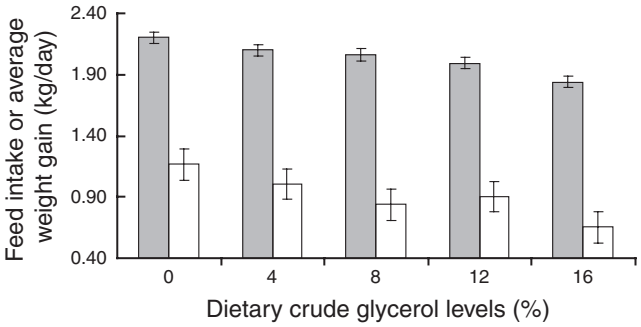

Fig. 1. The influence of increasing dietary levels of crude glycerol on average daily feed intake (ADFI) and average daily gain (ADG) in finishing pigs for the first week of the trial. Data are least-squares means (±s.e.). ADFI (shaded bars); *P*-values: overall: $P < 0.001$; linear: $P < 0.001$. ADG (open bars); *P*-values: overall: $P = 0.14$; linear: $P = 0.015$.

Blood analysis

The measured plasma concentrations of glycerol, glucose and NEFAs are given in Table 4. An interaction ($P < 0.05$) occurred between diets and sampling week for plasma glycerol, so data were then analysed by sampling week. There was no effect of diet on plasma glycerol levels when pigs were sampled in the second week of the experiment ($P > 0.05$), although there was a tendency ($P = 0.108$) for a linear rise in plasma glycerol levels as dietary glycerol levels increased. When plasma glycerol levels were determined one week before slaughter, diet influenced plasma glycerol levels ($P = 0.008$; linear: $P = 0.001$; quadratic: $P = 0.109$). Plasma levels of glucose or NEFAs were not influenced by dietary treatments.

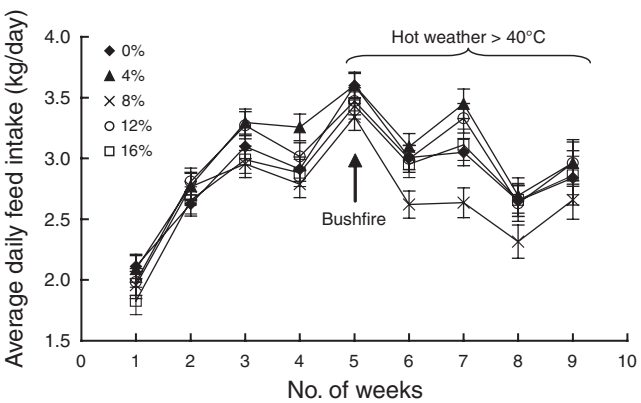

Fig. 2. The effects of increasing dietary levels of crude glycerol (0–16%) on average daily feed intake for each week of the trial. Data are least-squares means (±s.e.). *P*-values: overall: $P < 0.001$; linear: $P < 0.001$.

Table 3. Least-squares means for performance traits of pigs fed increasing levels of crude glycerol
Values followed by different letters are significantly different at $P = 0.05$

	Crude glycerol (%)					Pooled	*P*-value		
	0	4	8	12	16	s.e.	Overall	Linear	Quadratic
n	15	12	12	12	12	–			
ADFI[A] (kg)	2.91ab	3.04a	2.70b	2.96a	2.84ab	0.033	0.026	0.56	0.75
ADG[B] (g)	1037	1015	906	994	943	15.5	0.12	0.17	0.34
FCR[C]	2.82	3.04	2.98	3.00	3.03	0.038	0.42	0.25	0.34
P2 (mm)	15.5	15.4	16.8	15.4	14.1	0.41	0.37	0.28	0.16

[A]Average daily feed intake.
[B]Average daily gain.
[C]Feed conversion ratio.

Table 4. Least-squares means for plasma concentrations of glycerol, glucose and non-esterified fatty acids (NEFA) in pigs fed increasing levels of crude glycerol
Values followed by different letters are significantly different at $P = 0.05$

	Crude glycerol (%)					Pooled	*P*-value		
	0	4	8	12	16	s.e.	Overall	Linear	Quadratic
n	15	12	12	12	12	–			
Week 2									
Glycerol (μmol/L)	36	43	46	42	49	2.2	0.41	0.11	0.61
Glucose (mmol/L)	4.9	5.1	5.1	4.7	5.0	0.07	0.44	0.55	0.95
NEFA (mmol/L)[A]	0.14 (0.11–0.18)	0.18 (0.14–0.23)	0.18 (0.14–0.22)	0.17 (0.13–0.21)	0.18 (0.14–0.23)	–	0.70	0.35	0.48
Week before slaughter									
Glycerol (μmol/L)	34a	44a	221ab	639bc	1163c	117.6	0.008	0.001	0.109
Glucose (mmol/L)	4.9	4.9	4.7	4.5	5.0	0.08	0.31	0.59	0.21
NEFA[A] (mmol/L)	0.08 (0.05–0.12)	0.09 (0.06–0.15)	0.07 (0.04–0.12)	0.06 (0.04–0.10)	0.06 (0.04–0.09)	–	0.58	0.14	0.66

[A]NEFA data are logarithmically-transformed before statistical analysis. Back-transformed values and 95%-confidence intervals are presented.

Meat quality measurements

There were no differences in pH, drip loss, cook loss, colour and shear force as indices of meat quality attributable to increased levels of dietary crude glycerol (Table 5).

Discussion

Chemical characteristics of crude glycerol

Data obtained for the crude samples showed a large variation in all parameters measured. The difference in colour of the obtained crude glycerol samples can probably be explained by differences in the pigments and other compounds from the feedstocks used for the production of biodiesel. Sodium or potassium is often added as a catalyst in biodiesel production, and the amounts of these salts remaining in the crude glycerol depends on the refining process used (Ma and Hanna 1999; Gerpen 2005) and can vary substantially. The large variation in the amount of these minerals in ash, in addition to variation in the other parameters such as pH and the MONG content, will have implications for the type and inclusion level of the crude

Table 5. Least-squares means for meat quality indices of pigs fed increasing levels of crude glycerol

	Crude glycerol (%)					Pooled	*P*-value		
	0	4	8	12	16	s.e.	Overall	Linear	Quadratic
n	15	12	11	12	12	–			
pH 45 min	6.30	6.28	6.16	6.21	6.27	0.033	0.74	0.62	0.28
Ultimate pH	5.47	5.46	5.51	5.49	5.48	0.016	0.93	0.64	0.76
Lightness	52.51	52.88	53.74	54.11	51.42	0.586	0.69	0.84	0.21
Redness	5.36	5.76	6.03	5.59	6.15	0.179	0.68	0.30	0.85
Yellowness	3.55	3.59	3.61	3.66	3.31	0.172	0.98	0.31	0.26
24-h drip loss (%)	5.6	6.4	6.9	6.3	6.2	0.33	0.83	0.82	0.23
Cooking loss (%)	34.6	34.1	34.9	33.3	34.0	0.25	0.40	0.31	0.95
Warner-Bratzler shear force (kg)	6.62	6.54	6.15	6.04	6.86	0.24	0.86	0.92	0.29

glycerol for pig diets and should be taken into account when diets are formulated.

Another potential problematic compound in crude glycerol is methanol. Methanol toxicity in humans is characterised by central nervous system depression, weakness, headache, vomiting, metabolic acidosis and optic disc oedema, with the clinical consequences being blindness and/or Parkinsonian-like motor disease (Dorman *et al*. 1993). Methanol concentration can vary widely according to the manufacturing processes and should be monitored. To our knowledge no specification for the use of crude glycerol (with methanol) in animal feed has been published. In Germany, it is recommended that crude glycerol should contain a maximum of 0.2% methanol (Anonymous 2007) whereas the United States Food and Drug Administration require that methanol should not exceed 0.015% in the final animal feed (FDA 2007). No pigs demonstrated clinical symptoms of methanol toxicity in the present study even though the diet with 16% glycerol would have contained 0.29% methanol, assuming that all methanol in the crude glycerol remained in the feed. However, the analyses of the diets for methanol indicate that a significant proportion of the methanol evaporated from the diets.

Physical properties of diets

A physical advantage of dietary inclusion of crude glycerol was that the diets were less dusty than the control diet. However, inclusion of crude glycerol influenced the flow characteristics of the diets. All diets readily flowed out of the mixer even though the diets containing 12 and 16% glycerol left the vertical auger in the mixer with a sticky coating. The diets containing 8, 12 and 16% glycerol all formed a firm aggregate within 24 h after mixing. The higher the glycerol content the harder was the aggregate. In the present experiment, the pigs were fed by hand so although it was quite easy to break up the aggregate, feeding mash diets under commercial conditions with a glycerol content above 5 or 6% would probably be difficult to feed from bulk bins or using an automatic dry feeding system. Based on the present experience, it is not possible to say how diets high in crude glycerol would behave if the diets were pelleted or used in a liquid feeding system, although recent data suggests that 12% crude glycerol incorporated into a pellet is achievable (Groesbeck *et al*. 2008).

Performance

There was an unexpectedly high variance in performance that at least, in part, was attributable to a period of very hot weather (>40°C) and a bush fire passing very near the research facility in the mid to latter part of the study. Nevertheless, the feed intakes observed during this period of hot weather are within the general range seen commercially in Australia. In the first week of the study, however, feed intake and subsequently average daily gain decreased linearly with increasing dietary crude glycerol levels indicating that the pigs had to adapt to the inclusion in the diet during the initial part of the experiment. It is, therefore, recommended that pigs be introduced gradually to diets containing crude glycerol to overcome any possible depression in performance.

Previous research with various species has shown contradictory growth performance results (Kerr *et al*. 2007). Kijora *et al*. (1995) conducted two experiments using only six individually housed pigs per treatment to test glycerol as a component in diets of growing-finishing pigs. In their first experiment, pigs fed diets containing 5 and 10% glycerol had increased daily weight gain; however, this was only significant for the pigs fed the diet with 10% glycerol. In the second experiment the effect of 0, 5, 10, 20 and 30% inclusion levels of glycerol was tested, with no significant improvement in performance being detected. However, adding 30% glycerol to the diet negatively impaired gain and feed conversion ratio. In a subsequent study the utilisation of 'crude' and 'pure' glycerol as a feed component was investigated (Kijora and Kupsch 1996). The control diet contained no glycerol and the five other diets were supplemented with either 5 or 10% glycerol. Feed intake and daily gain of pigs in the growing period were elevated in all groups that received glycerol compared with the control group, although the authors stated that this result might have been a result of suboptimal protein content in the grower control diet (Kijora and Kupsch 1996). There was no adverse effect of glycerol during the finishing period where protein content of the diets was adequate. In an experiment looking at the inclusion of 10% glycerol in the diet, pigs fed the basal diet plus 10% glycerol had the highest feed intake and daily weight gain in the growing period, but no difference in daily gain was detected over the entire growing-finishing period (Kijora *et al*. 1997). In contrast, Mourot *et al*. (1994), using 10 pigs per treatment, reported a tendency towards reduced daily gain and an inferior feed conversion ratio of feeding 5% crude glycerol (based on tallow or rapeseed oil) to finishing pigs. However, recent studies evaluating glycerol in diets for nursery pigs indicated that up to 6% dietary glycerol had no detrimental effect on performance (Groesbeck *et al*. 2008; Hinson *et al*. 2008), and Lammers *et al*. (2008*c*) reported no differences in pig performance between weaning and slaughter when 10% crude glycerol was included in the diet.

Metabolism

The absence of any differences in plasma glycerol levels when the animals were sampled in the second week of the experiment suggests that all dietary glycerol had been metabolised, which is in accordance with findings by Mourot *et al*. (1994) when dietary levels of 5% glycerol were fed to pigs. Kijora and Kupsch (1996) observed that the concentration of glycerol in plasma collected 12 h after feeding was elevated in slaughter-weight pigs fed either 5 or 10% glycerol to approximately the same levels as in the present study. In accordance, when pigs were bled 30 min after a second daily feeding, Kijora *et al*. (1995) found an increase in plasma glycerol levels when the dietary glycerol levels increased from 0% up to 30%. However, as these pigs were bled just after being fed, the measured plasma glycerol levels by Kijora *et al*. (1995) were 1000-fold higher than the levels measured in our experiment.

The significantly higher plasma glycerol measured in our study when pigs were bled one week before slaughter could indicate a shift in pig metabolism in such a way that the younger pig was better able to metabolise glycerol, and/or key liver enzymes such as glycerol kinase involved in the metabolism of glycerol became saturated as the duration of feeding, and hence total intake of glycerol increased. Glycerol absorbed across the

gastrointestinal tract can be converted to glucose in the liver, and if the gluconeogenetic capacity of the liver is exceeded, excess glycerol is most likely excreted in the urine (Kijora *et al*. 1995). Plasma glycerol levels in the present study increased markedly with more than 4% dietary crude glycerol (Table 4), which suggests a potentially lower energy supply to the pig because energy as the gluconeogenic precursor glycerol could be lost in the urine. This, in turn, could have contributed to the variability in the performance data observed, although we were not able to measure glycerol in the urine or could we find any correlation between increased blood glycerol levels and decreased pig performance (data not shown). Nevertheless, data from Lammers *et al*. (2008*a*) suggests that finishing pigs (99 kg) can metabolise glycerol at levels up to 10% in the diet, although they noted a reduction in the metabolisable energy value for starter pigs fed 20% crude glycerol (86.95% glycerol).

Plasma glucose and NEFAs levels measured in fasted animals were not markedly affected by glycerol treatment. The effect of sampling week on the plasma levels of NEFAs could be due to age-related differences in fat metabolism between the two different sampling times.

Meat quality

In accordance with the present investigation, Kijora *et al*. (1995) and Kijora and Kupsch (1996) also failed to detect any differences in meat quality indices in finishing pigs fed glycerol. More recently, Lammers *et al*. (2008*c*) reported that pigs could be fed up to 10% crude glycerin with no negative effects on carcass composition or meat quality, although 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 *v*. 5.57, respectively). Moreover, fatty acid profile of the loin from pigs fed 10% crude glycerin had less linoleic acid ($P < 0.01$) and more eicosapentaenoic acid ($P = 0.02$) than pigs fed the 0 or 5% crude glycerin diets. In contrast, Mourot *et al*. (1994) reported reduced drip and cooking losses in meat from pigs fed 5% added glycerol. It is difficult to express reason for these differences, but they are most likely related to factors including diet type, pig genotype, and different post-slaughter conditions in the abattoir.

Conclusions and implications

The results of this experiment indicate that crude glycerol could be included in finishing pig diets without adversely affecting the growth performance or meat quality of pigs. However, blood glycerol levels became elevated after prolonged feeding that may reduce the efficiency of glycerol when used as an energy source for pigs. Furthermore, diets that contained more than 8% glycerol formed a firm aggregate within 24 h of mixing that presented some feeding difficulties, which most likely limits crude glycerol to dietary levels less than this in mash diets. Crude glycerol may play a role in the pig industry by supplying energy at a more cost-effective price than competing energy ingredients; however, a shadow-pricing exercise is necessary to ascertain whether glycerol can be economically included in current diets. In addition, the levels of residues, such as methanol and ash, and other parameters including pH, moisture and the MONG content in the crude glycerol should be monitored. This is to prevent excessive amounts of these compounds and (or), in the case of MONG that is most likely fatty acids, make an allowance for its energy contribution in the diet formulation.

Acknowledgements

The Australian Cooperative Research Centre for an Internationally Competitive Pork Industry (Pork CRC) financially supported this research. The authors wish to express their gratitude to staff at the Medina Research Station, Department of Agriculture and Food, for care of the animals and help with collection of data, and to the Australian biodiesel manufacturers for donation of glycerol samples.

References

Anonymous (2007) Positive list for straight feedingstuffs (feed materials). Available at www.q-s.info/fileadmin/download/handbuch/futtermittel/en/positive_list_for_feed_material_6_version.pdf [Verified 2 December 2008]

AOAC (1997) 'Official methods of analysis.' 16th edn. (Association of Official Analytical Chemists: Washington, DC)

Bouton PE, Harris PV, Shorthose WR (1971) Effect of ultimate pH upon the water holding capacity and tenderness of mutton. *Journal of Food Science* **36**, 435–439. doi: 10.1111/j.1365-2621.1971.tb06382.x

Channon HA, Baud SR, Kerr MG, Walker PJ (2003) Effect of low voltage electrical stimulation of pig carcasses and ageing on sensory attributes of fresh pork. *Meat Science* **65**, 1315–1324. doi: 10.1016/S0309-1740(03)00052-4

Dorman DC, Dyy JA, Nassise MP, Ekuta J, Bolon B, Medinsky MA (1993) Acute methanol toxicity in minipigs. *Fundamental and Applied Toxicology* **20**, 341–347. doi: 10.1006/faat.1993.1044

Dozier WA III, Kerr BJ, Corzo MT, Kidd TE, Weber K, Bregendahl K (2008) Apparent metabolizable energy of glycerin for broiler chickens. *Poultry Science* **87**, 317–322. doi: 10.3382/ps.2007-00309

FDA (2007) US Food and Drug Administration. Food additives permitted in feed and drinking water of animals. Methyl esters of higher fatty acids. Available at http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.640[Verified 13November 2008]

Gerpen JV (2005) Biodiesel processing and production. *Fuel Processing Technology* **86**, 1097–1107. doi: 10.1016/j.fuproc.2004.11.005

Groesbeck CN, McKinney LJ, DeRouchey JM, Tokash MD, Goodband RD, Dritz SS, Nelssen JL, Duttlinger AW, Fahrenholz AC, Behnke KC (2008) Effect of crude glycerol on pellet mill production and nursery pig growth performance. *Journal of Animal Science* **86**, 2228–2236. doi: 10.2527/jas.2008-0880

Hinson R, Ma L, Allee G (2008) Use of glycerol in nursery pig diets. *Journal of Animal Science* **86**, 46 [e-Suppl. 3].

Honikel KO (1998) Reference methods for the assessment of physical characteristics of meat. *Meat Science* **49**, 447–457. doi: 10.1016/S0309-1740(98)00034-5

Kerr BJ, Dozier WA III, Bregendahl K (2007) Nutritional value of crude glycerin for nonruminants. In 'Proceedings of the 68th Minnesota nutrition conference: modern concepts in livestock production for 2007'. pp. 220–234. (University of Minnesota: Minneapolis, MN)

Kijora C, Kupsch RD (1996) Evaluation of technical glycerols from 'biodiesel' production as a feed component in fattening of pigs. *Fett-Lipid* **98**, 240–245. doi: 10.1002/lipi.19960980703

Kijora C, Bergner H, Kupsch RD, Hagemann L (1995) Glycerol as a feed component in diets of fattening pigs. *Archives of Animal Nutrition-Archiv Fur Tierernahrung* **47**, 345–360.

Kijora C, Kupsch RD, Bergner H, Wenk C, Prabucki AL (1997) Comparative investigation on the utilization of glycerol, free fatty acids, free fatty acids in combination with glycerol and vegetable oil in fattening of pigs. *Journal of Animal Physiology and Animal Nutrition-Zeitschrift Fur Tierphysiologie Tierernahrung Und Futtermittelkunde* **77**, 127–138.

Lammers PJ, Kerr BJ, Weber TE, Dozier WA III, Kidd MT, Bregendahl K, Honeyman MS (2008*a*) Digestible and metabolizable energy of crude glycerol for growing pigs. *Journal of Animal Science* **86**, 602–608. doi: 10.2527/jas.2007-0453

Lammers PJ, Kerr BJ, Honeyman MS, Stalder K, Dozier WA III, Weber TE, Kidd MT, Bregendahl K (2008*b*) Nitrogen-corrected apparent metabolizable energy value of crude glycerol for laying hens. *Poultry Science* **87**, 104–107. doi: 10.3382/ps.2007-00255

Lammers PJ, Kerr BJ, Weber TE, Bregendahl K, Lonergan SM, Prusa KJ, Ahn DU, Stoffregen WC, Dozier WA III, Honeyman MS (2008*c*) Growth performance, carcass characteristics, meat quality, and tissue histology of growing pigs fed crude glycerin-supplemented diets. *Journal of Animal Science* **86**, 2962–2970. doi: 10.2527/jas.2008-0972

Ma F, Hanna MA (1999) Biodiesel production: a review. *Bioresource Technology* **70**, 1–15. doi: 10.1016/S0960-8524(99)00025-5

Mourot J, Aumaitre A, Mounier A, Peiniau P, Francois AC (1994) Nutritional and physiological effects of dietary glycerol in the growing pig. Consequences on fatty tissues and post mortem muscular parameters. *Livestock Production Science* **38**, 237–244. doi: 10.1016/0301-6226(94)90175-9

Thompson JC, He BB (2006) Characterization of crude glycerol from biodiesel production from multiple feedstocks. *Applied Engineering in Agriculture* **22**, 261–265.

Manuscript received 28 July 2008, accepted 15 September 2008