Effect of crude glycerol from South African biodiesel production on growth, carcass characteristics and pork quality of pigs

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Abstract

A study was carried out to evaluate the effects of dietary inclusion of crude glycerol as a partial replacement of maize at 50 g/kg and 100 g/kg in pig grower diets. Three experimental diets were formulated, a control (CN) and two diets containing 50 g/kg (low glycerol (LG)) and 100 g/kg (high glycerol (HG)) glycerol. The experimental diets were fed in a growth and carcass evaluation study to 60 Large White crossbred pigs (30 males and 30 females) weighing 21 ± 1.6 kg. There were no differences between the glycerol inclusion levels in daily gain, feed intake and feed conversion ratio and carcass traits of the pigs. Gilts had a higher dressing percentage and lower backfat thickness than boars. They also had shorter carcasses and a lower drip loss percentage than boars. There were no differences in thawing, drip, evaporation and total cooking losses of pork loin chops between treatments. It was concluded that crude glycerol can be included up to 100 g/kg in grower pig diets without any negative effects on pig performance.

Keywords: Energy, digestibility, fatty acids, biofuel, cooking characteristics
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Introduction

Biodiesel is produced from fats and oils by transesterification. In South Africa, sunflower supplies 82% of edible oil because it is well adapted to the underutilized drier areas and marginal soils in the country. It has therefore been considered a potential feedstock for biodiesel production since there is potential to increase its production. The process of esterification yields about 79 g of crude glycerol as main co-product for each litre of biodiesel, which can be used in pig feeding (Lammers *et al.*, 2007; Groesbeck *et al.*, 2008) to offset high feed prices.

The utilization of the crude glycerol obtained as a feed ingredient, however, is complicated because its composition varies widely, depending on the feedstock, type of catalyst, transesterification efficiency, recovery efficiency of the biodiesel, other impurities in the feedstock, and whether the methanol and catalysts were recovered (Yang *et al.*, 2012). A survey of 11 Australian biodiesel production plants revealed that glycerol concentrations ranged from 38% to 96% and varied in quality with large variations in pH, moisture, ash and methanol content (Hansen *et al.*, 2009). In a similar study on glycerol from various biodiesel producing plants in the United States, Kerr *et al.* (2009) and Dozier *et al.* (2011) reported varying levels of glycerol (50% to 88%). The gross energy content of various crude glycerol samples analysed by Kerr *et al.* (2009) varied between 13.27 MJ/kg and 25.19 MJ/kg, depending on the glycerol and fatty acid content. This makes it imperative to evaluate South African crude glycerol sources before they can be used for feeding pigs.

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The inclusion of crude glycerol in pig diets from sources with at least 86% glycerol (acidulated glycerol) gave optimum utilization up to a level of 50 g/kg, while higher levels of more than 100 g/kg resulted in lower metabolizable energy values of the diets, because the enzyme system in the pigs became saturated and excess glycerol was excreted via the urine (Kijora *et al.*, 1995; Lammers *et al.*, 2008a). Glycerol inclusion in the diet of finishing pigs was also shown to improve the water-holding capacity of pork (Mourot *et al.*, 1994). Little work has been done to evaluate the performance of pigs fed diets containing crude glycerol, which is expected to contain more energy owing to higher levels of residual free fatty acids.

Crude glycerol not only has the potential to lower feed costs because of its energy content, but may have additional value by limiting drip loss and cooking loss (Mourot *et al.*, 1994). These aspects both warrant investigation using local crude glycerol. It was therefore hypothesized that South African crude glycerol could replace up to 100 g/kg of maize as an energy source in maize-soybean oilcake meal-based grower diets for pigs without affecting growth and carcass traits while enhancing the cooking qualities of the pork.

Materials and Methods

Crude glycerol (Table 1) was obtained from the Biotech biodiesel plant at Bainsvlei in Bloemfontein, some 500 km south of Pretoria. Diets were formulated to contain 0 g (control (CN)), 50 g (low glycerol (LG)) and 100 g (high glycerol (HG)) crude glycerol/kg (Table 2), and were used in growth performance trial. The three diets were formulated to be isoenergetic (14 MJ digestible energy/kg) and isonitrogenous (180 g crude protein/kg) and to meet or exceed the recommended requirements for growing pigs under South African conditions (Viljoen, 1993). The digestibility and growth trial procedures were approved by the Ethics Committee of the ARC-API (ref number: APIEC08/11).

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Table 1 Chemical composition of crude glycerol (DM basis)

Component	Quantity	Method of analysis
Fatty acids (g/kg)	557.1	AOAC (1996)
Glycerol (g/kg)	442.8	Gas chromatography
[a]GE (MJ/kg DM)	15.3	Adiabatic bomb calorimeter
Potassium (mg/kg)	174	ICP-OES[b]
Sodium (mg/kg)	73	ICP-OES
Calcium (mg/kg)	27.9	ICP-OES
Magnesium (mg/kg)	8.53	ICP-OES
Copper (mg/kg)	2.38	ICP-OES
Zinc (mg/kg)	1.29	ICP-OES
Phosphorus (mg/kg)	10.9	ICP-OES
Sulphur(mg/kg)	40.53	ICP-OES
Lead (mg/kg)	0.43	ICP-OES

[a]Assuming 40 MJ gross energy (GE)/kg from fatty acids and 18 MJ/kg from glycerol the calculated GE content of the product is 15.27 MJ/kg, which coincides with the 15.29 MJ/kg measured.
[b] Inductively coupled plasma optical emission spectrometry.

Sixty Large White x Landrace crossbred pigs (30 males and 30 females) aged 60 days were sourced from the Animal Production Institute stock, and weighed 23 ± 1.6 kg. They were allocated to one of the three experimental diets (Table 2) in a 3 x 2 (treatment x sex) factorial experiment design. The experimental period lasted eight weeks and the pigs were slaughtered at a target liveweight of 65 kg. The pigs were housed in same-sex pairs per pen as an experimental unit in an environmentally controlled house with a space

allowance of 0.8 m^2 per pig. The pigs were fed *ad libitum* and were weighed individually at the start of the trial and weekly until the end of the trial. Water was available at all times through drinking nipples. Feed residues were weighed weekly to determine feed intake (weekly and cumulative) and feed conversion ratio per pen. At eight weeks a standard pre-slaughtering procedure was followed, which included an *ante-mortem* inspection and a two hour rest for the pigs before slaughter. Prior to slaughter, the pigs were electrically stunned with an electrical stunner set at 220 V and 1.8 A with a current flow for 6 seconds and exsanguinated within 10 seconds of stunning.

Table 2 Composition of experimental diets; control (CN), 50 g/kg (LG) and 100 g/kg of crude glycerol (HG) fed to growing pigs from 20 to 65 kg (g/kg) (as-fed basis)

Ingredients (g/kg)	Control	LG diet	HG diet
Maize	697.5	599.7	568.3
Wheat bran	10.2	9.1	8.3
[a]Crude glycerol	-	50.0	100
Soybean meal oilcake	164.6	203.2	180.6
Full-fat soya	90	100	100
Limestone	13.0	13.0	5.6
Salt	4.0	4.0	4.0
L-Lysine HCl	11.0	11.0	11.3
Monocalcium phosphate	5.8	5.4	18.0
[b]Vitamin-mineral premix	4	4	4
Calculated nutrient composition (on an as-fed basis)			
Dry matter (g/kg)	910.8	910.3	910.1
Crude protein (g/kg)	190.4	190.1	200.1
Energy (MJ DE /kg)	14.0	13.8	14.0
Crude fibre (g/kg)	30.4	30.7	30.1
Calcium (g/kg)	7.5	9.2	8.8
Phosphorus (g/kg)	4.7	5.0	7.6

[a] The crude glycerol was obtained from Biotech biodiesel plant in Bainsvlei.
[b] Provided the following per kg of diet: 6500 IU vit. A; 1200 IU vit. D_3; 40 IU vit. E; 2 mg vit. K_3; 1.5 mg vit. B_1; 4.5 mg vit. B_2; 0.03 mg vit. B_{12}; 2.5 mg vit. B_6; 25 mg niacin; 12 mg calcium pantothenate; 190.5 mg choline; 0.6 mg folic acid; 0.05 mg biotin; 40 mg Mn; 100 mg Zn; 125 mg Cu; 1 mg I; 100 mg Fe; 0.3 mg Se.

Dehairing and evisceration followed and warm carcass weights were taken about half an hour after evisceration. Backfat thickness was also taken on warm carcasses at P1 (45 mm from mid-section) between the second and third-last rib on the left side of the pigs, using the Hennessy Grading Probe® (Hennessy Grading Systems, New Zealand) within 45 minutes of slaughter. After an overnight chill storage at an average temperature of 4 ºC, cold carcass weights were determined. Carcass length was taken on split carcasses by measuring from the pelvic bone to the first thoracic vertebra using a measuring tape. Chiller shrinkage was calculated for carcasses by determining the weight loss after the overnight chill and expressing it as a percentage of warm carcass weight.

Eighteen loin samples (six per treatment), averaging 1.35 kg, were obtained from the left sides of randomly selected carcasses of gilts after an overnight chill of 4 ºC, and were immediately frozen at −20 °C until analysis. Six frozen pork loin samples of each treatment were removed from the freezer and cut into 3 cm chops. The frozen chops were weighed, individually vacuum packed to prevent moisture loss, and placed individually on oven trays in the cold room at 4 ºC for 24 h to thaw. The chops were reweighed after thawing. The thawing loss was calculated as the weight difference between the frozen and thawed loin

chops, expressed as a percentage of the frozen weight of the loin chops. The samples were subsequently removed from the racks, placed in pre-weighed roasting pans, and roasted open in Miele® ovens set on 200 °C fan grill. The chops were roasted until the temperature in the geometric middle reached 75 - 77 ºC, as measured with a hand-model digital probe (Kane-May, model 1012) equipped with a K-type thermocouple, after which the samples were weighed. The total cooking loss of samples was calculated as the difference between the thawed raw and cooked weights, expressed as a percentage of the thawed-raw weight. The weight of the pan with leftover liquid was also taken to determine the weight of the leftover liquid. Drip loss was calculated as the weight of liquid left in the pan expressed as a percentage of the thawed-raw weight of the pork loin sample. The residual fluid was poured into measuring cylinders and left to separate into stock and fat, which were expressed as percentage of the total fluid. Evaporation loss was calculated as the difference between the total cooking loss and drip loss. Yield per edible portion percentage was expressed as total cooking loss percentage subtracted from 100%. No water was added during the cooking process.

Calculations were performed according to the guidelines of the American Meat Science Association (1978). Cooking losses, drip losses and evaporation losses were determined as described by Schönfeldt *et al.* (1997). Feed samples were oven-dried at 60 ºC for 24 h to determine dry matter (DM). The samples were ground using a centrifugal force mill (Retch Ultra-centrifugal Miller, F. Kurt Retch, Haan, Germany) and the Kjeldahl nitrogen crude protein (N x 6.25) (CP) was determined from the standard methods of AOAC (1990). Gross energy (GE) was measured by complete combustion using an adiabatic bomb calorimeter (MC-1000 MK 2, Energy Instrumentation, South Africa). Crude fibre (CF) was determined according to the methods of Van Soest (1963).

The growth performance and carcass traits were analysed using GLM procedures of SAS (2004). Diets and sex were used as the main effects in a two-way analysis of variance at a significance level of $P < 0.05$ and sex by treatment interactions were also determined. If the main effect was significant, the Fisher LSD-test was applied to determine the direction of the differences between the mean values.

Analysis of variance on thawing losses, total cooking losses, drip losses, and evaporation loss percentages was also done using the Genstat for Windows (2003). The three treatments were used as the main effect in one-way analyses of variances at a significance level of 95% ($P \leq 0.05$).

Results

Table 3 Least square means of final weight, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of male (n = 30) and female (n = 30) grower pigs fed the control (CN), low glycerol (LG) and high glycerol (HG) diets from 23 to 65 kg

Diet	Sex	Final weight (kg)	ADG (kg/d)	ADFI (kg/d)	FCR
Control	Gilts	63.6	0.8	1.8	2.3
	Boars	63.3	0.8	1.7	2.1
LG	Gilts	62.5	0.8	1.8	2.3
	Boars	65.6	0.9	1.7	1.9
HG	Gilts	61.7	0.8	1.7	2.1
	Boars	64.2	0.9	1.7	1.9
P-value	Tmt	0.634	0.645	0.520	0.641
	Sex	0.063	0.061	0.346	0.0048
	Tmt x sex	0.310	0.287	0.536	0.146
SEM		1.13	0.023	0.044	0.054

[a-b] Means in the same column without superscripts do not differ ($P > 0.05$).
SEM: standard error of means; Tmt: treatment.

Final weights, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of pigs fed the diets are shown in Table 3. There were no interactions (P >0.05) between gender and diet for any of the parameters. There were no differences in the final weights, ADG, ADFI and FCR between the treatments (P >0.05). There were no differences between sexes within treatments in final weights, ADG and ADFI (P >0.05). Boars had a lower (P <0.01) FCR compared with gilts.

Effects of treatments on carcass traits are shown in Table 4. There were no interactions (P >0.05) between gender and treatment for any of the parameters. There were no differences (P >0.05) in warm and cold carcass weights, dressing percentage, carcass lengths, backfat thickness and drip loss percentage between the treatments. There were no sex differences within treatments in warm and cold carcass weights (P >0.05). However, gilts had higher (P <0.01) dressing percentage and lower backfat (P = 0.01) and shorter carcasses (P <0.05) and lower chiller shrink percentage (P <0.05) than the boars.

Table 4 Least square means of carcass traits and sex differences of male (n = 30) and female (n = 30) pigs fed the control (CN), 50 g/kg (LG) and 100 g/kg (HG) glycerol diets from 23 to 65 kg

Diets	Sex	Warm carcass wt (kg)	Cold carcass wt (kg)	Dressing %	Carcass length (mm)	Backfat (mm)	Chiller shrink %
Control	Gilts	48.7	47.6	76.8	686	9.0	2.3
	Boars	48.2	46.8	76.6	683	12.3	3.1
LG	Gilts	48.4	47.4	77.2	684	10.8	2.2
	Boars	50.8	48.9	76.9	705	11.6	3.7
HG	Gilts	48.7	47.4	78.6	680	11.2	2.7
	Boars	48.8	47.9	76.3	686	12.5	2.4
P-value	Tmt	0.57	0.70	0.34	0.21	0.44	0.67
	Sex	0.48	0.68	0.03	0.03	0.02	0.05
	Tmt x Sex	0.43	0.60	0.07	0.23	0.36	0.11
SEM		0.45	0.46	0.23	0.28	0.39	0.18

Means in the same column with different superscripts differ ($P \leq 0.05$).
Tmt: treatment; SEM: standard error of means.

Data on the cooking quality of pork from pigs fed the experimental diets is shown in Table 5. There were no differences (P >0.05) in the percentage thawing loss, percentage drip loss, percentage evaporation loss and percentage total cooking losses of pork loin chops between the treatments.

The percentages of fat in the drip for the CN, LG and HG groups were 46.5%, 46.5% and 46.3%, respectively.

Discussion

The major shortcoming of using crude glycerol is that it has numerous impurities such as alcohol residues (methanol) and lead (Pb), which can potentially harm the animal. There were no detectable levels of alcohol residues, and these have been reported to evaporate during processing and storage (Doppenberg & Van der Aar, 2007). The Pb levels in the biodiesel of 0.85 mg/kg were below the safe maximum levels of 5 mg/kg stipulated in European feedstuffs (Directive 2002/32/EC). The other concern is that the salts (sodium chloride/sodium sulphate), non-esterified fatty acids (linoleic and linolenic acids) and water (Hansen *et al.*, 2009) will not be uniformly distributed at any given time and this will have implications when one is mixing the diets.

Table 5 Least square mean values of thawing loss %, drip loss %, evaporation loss % and total cooking loss % of pork loin chop samples from female pigs (n = 6) fed control, 50 g/kg (LG) and 100 g/kg (HG) glycerol diets

Diets	Thawing loss %	Drip loss %	Evaporation loss %	Total cooking loss %
Control	2.95	7.43	18.00	25.43
LG	2.82	7.68	18.48	26.15
HG	2.29	8.27	17.83	26.10
P-value	0.137	0.732	0.481	0.691
SEM	0.626	2.027	1.021	1.724

Means in the same column without superscripts do not differ ($P > 0.05$).
SEM: standard error of means.

In the current study, the glycerol was retrieved manually from 200-litre drums and attempts were made to ensure that the glycerol was mixed thoroughly before diet formulation. However, there was no evidence of disparities in nutrient concentrations (see Table 1) in the diets that could be attributed to lack of uniformity in the crude glycerol. It is therefore critical to analyse each consignment of crude glycerol before formulating the diet.

The observation that the level of crude glycerol did not affect feed intake, ADG and FCR is similar to other studies that used acidulated crude glycerol (Kijora *et al.*, 1995; Lammers *et al.*, 2007; Lammers *et al.*, 2008b; Hansen *et al.*, 2009). Groesbeck *et al.* (2008), however, reported a higher numerical average daily feed intake by nursery pigs and a higher ADG with 6% acidulated glycerol inclusion levels. The inclusion of pure glycerol at 5% and 10% levels had no effect on ADG and FCR in growing pigs (Mourot *et al.*, 1994; Kijora *et al.*, 1995). Since voluntary feed intake is affected by palatability (Kijora *et al.*, 1995; Sterk *et al.*, 2008), gut fill (Lange *et al.*, 2002) and energy density (Petty *et al.*, 2002), among other factors, it is likely that the crude glycerol did not affect palatability sufficiently to increase feed intake. The differences in FCR between sexes in the LG diet are similar to findings by Hansen & Lewis (1993) in which boars had lower feed conversion ratios than gilts. Beattie *et al.* (1999) reported that gilts consume more, while growing more slowly with age.

There were no differences in either the warm or the cold carcass weights between the treatments and between sexes; results that are similar to other findings using unrefined glycerol (Lammers *et al.*, 2007; Lammers *et al.*, 2008b) and pure glycerol (Mourot *et al.*, 1994; Kijora *et al.*, 1995), between gilts and boars at 110 kg slaughter weight (Nold *et al.*, 1997) and at weights ranging from 92 kg to 131 kg (Beattie *et al.*, 1999). Gilts, however, had a higher dressing percentage than boars, as was also reported by Hansen & Lewis (1993). There were no differences in carcass length among treatments, even though carcass length of the boars was longer than that of gilts in the 5% diet. Hansen & Lewis (1993) also reported longer carcass lengths of boars than gilts slaughtered at 105 kg. The results suggested normal body development of pigs on glycerol diets.

There were no differences in backfat thickness between the treatments, and the high coefficient of variation (>26%) deems that caution should be exercised in drawing conclusions. The results, however, agree with those of Hansen *et al.* (2009), who used glycerol inclusion rates of 16% in diets of growing-finishing pigs. Mourot *et al.* (1994) reported no differences in backfat thickness of castrated boars as a result of pure glycerol inclusion of 5%. Surprisingly, in the current study, boars were fatter than gilts in the control group, which contradicts results from other studies. Gilts are usually fatter on normal diets (McPhee & Daniels, 1991; Nold *et al.*, 1997; Beattie *et al.*, 1999; Magowan & McCann, 2006). This may have been owing to lower slaughtering weights of pigs in the current study. In the other studies cited, the pigs were slaughtered at over 90 kg, whereas in the current study they were slaughtered at an average weight of 63.5 kg. McPhee & Daniels (1991) reported thicker backfat of boar carcasses than those of gilts at 25 kg liveweight, a scenario which changed at higher liveweights. As pigs grow in liveweight, there is an increase in backfat thickness of the two sexes, but this increase is greater in gilts than boars (McPhee &Daniels, 1991). Similarly, Beattie *et al.* (1999) reported a significant increase in subcutaneous fat of gilts with increasing carcass weight. This

occurs as gilts reach their lean deposition peak at a lower liveweight and start to deposit subcutaneous fat (Beattie *et al.*, 1999). Another possible reason for the fatter boars could be differences in seasons, as reported by Siers (1975) that boars had similar backfat thicknesses to gilts in a spring experiment, but were leaner in an autumn experiment. Lammers *et al.* (2007) reported no sex by treatment interaction in carcass traits of castrated boars and gilts. This was also the case in the current study with gilts and boars, even though the pigs were slaughtered at lower body weights compared with pigs in the study by Lammers *et al.* (2007) that were slaughtered at weights averaging 133 kg.

The similarity in the thawing loss percentage, total cooking loss percentage, drip loss percentage and evaporation loss percentage of pork loin chop samples among the three treatments means that consumers will not get a different pork product if crude glycerol is used in diets. While the drip loss may be confounded by variation in the fat content of the drip, results showed that there were no differences between treatments in the composition of the drip. However, while Lammers *et al.* (2008b) and Hansen *et al.* (2009) reported no effects of unrefined glycerol on meat quality up to 10% and 16%, respectively, Mourot *et al.* (1994) reported a reduced drip loss of both *longissimus dorsi* and *semimembranosus* muscle samples, although in their study the castrated boars were fed diets with pure glycerol. No definitive conclusions can be reached from results from the current study on drip loss because of the large coefficient of variation (>25%) of the means. It could be a reflection of the relatively few replications that were used in the study. In an earlier study, Nold *et al.* (1999) reported that boars had a higher drip loss when slaughtered at 100 kg, while the drip loss was similar at 110 kg, implying that weight at slaughter plays an important role. In contrast, Beattie *et al.* (1999) reported that no differences were observed between gilts and boars in drip loss percentages of *longissimus dorsi* muscle samples, although cooking losses of muscle samples from boars were higher than those of gilts.

Conclusions

The study shows that South African crude glycerol (44% glycerol; 56% fatty acids), a by-product of sunflower oil biodiesel production, obtained from Bainsvlei, can be used in the formulation of diets of growing pigs at an inclusion level of 100 g/kg to offset high feed costs. It had no negative effects on the growth of pigs, carcass characteristics and cooking characteristics of the pork. Further studies are needed to determine whether higher levels can be used and to establish their possible effects on meat quality in terms of drip loss.

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