

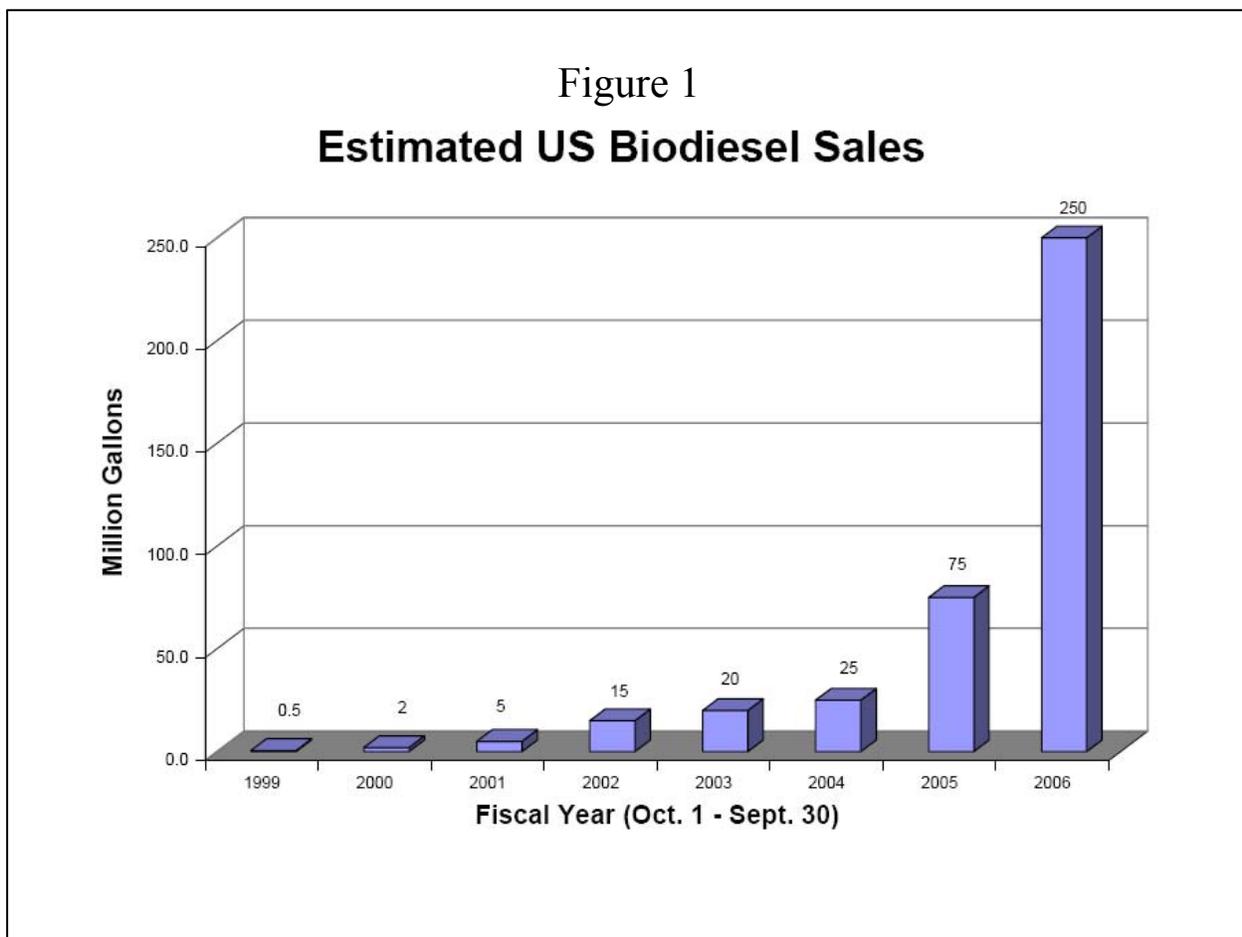
NUTRITIONAL VALUE OF CRUDE GLYCERIN FOR NONRUMINANTS

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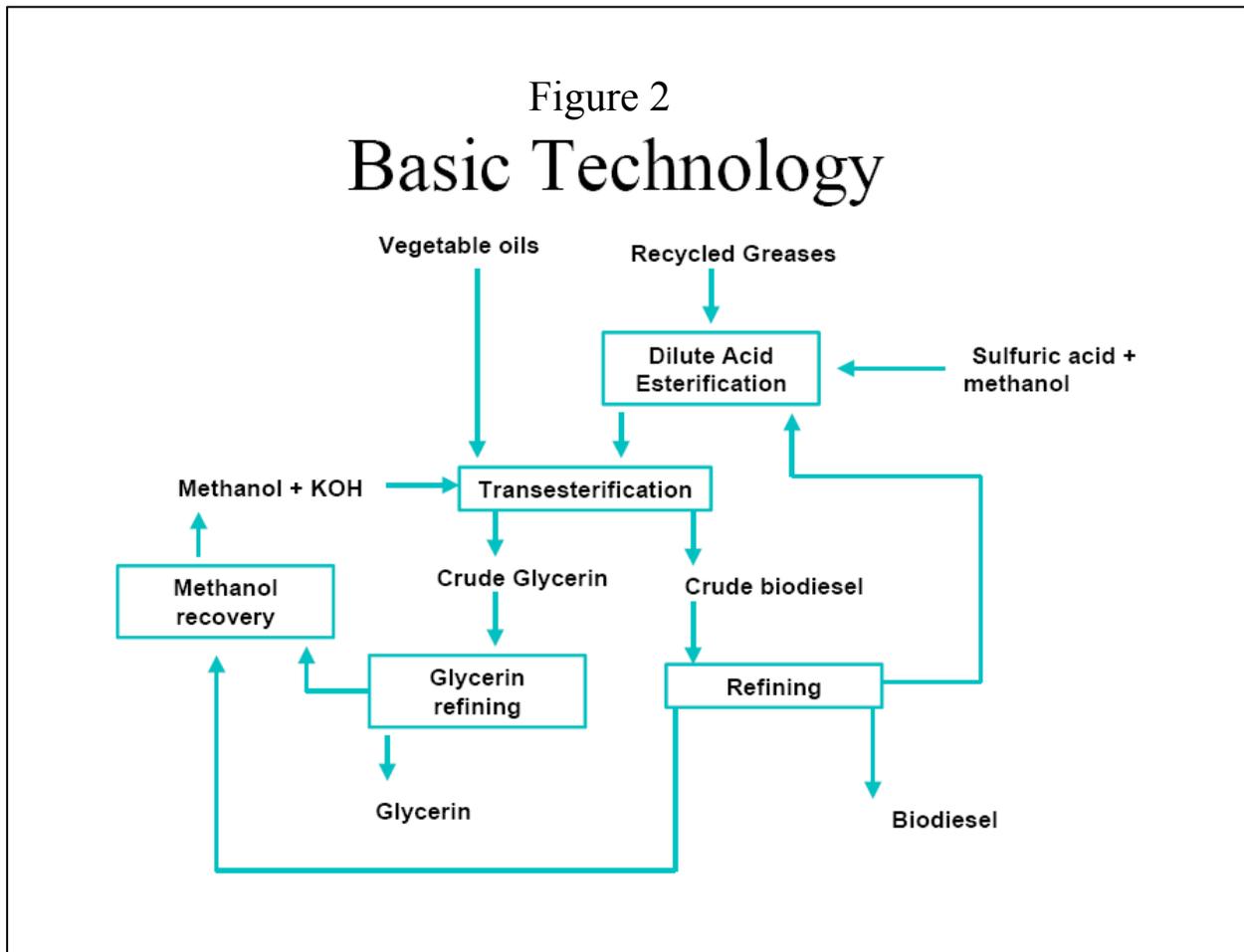
INTRODUCTION

In 2004, the United States consumed 140 billion gallons of gasoline and 40 billion gallons of diesel for on-road transportation (Annual Energy Outlook, 2007; <http://www.eia.doe.gov/oifa/aeo/>). In an effort to reduce dependence on petroleum-based fuel products and reduce their negative impact on the environment (Hill et al., 2006), production of biofuels from renewable energy sources has experienced explosive growth. Like the rapid expansion of ethanol production (projected 4.8 billion gallon production in 2006, Iowa Corn Promotion Board, 2007; <http://www.iowacorn.org>), biodiesel production has also rapidly expanded, albeit a smaller production volume, to its current production level of 250 million gallons (Figure 1; National Biodiesel Board, 2007; <http://www.nbb.org>).



Biodiesel can be produced by a variety of esterification technologies, using new or used vegetable oils and animal fats as the initial feedstock. In general, oils and fats are filtered and preprocessed to remove water and contaminants, followed by mixing with an alcohol (usually

methanol) and a catalyst (sodium or potassium methylate). This causes the oil molecules (triglycerides) to be broken apart into methylesters and glycerin, which are then separated from each other and purified (Figure 2, Alternative Fuels Data Center, 2007, <http://www.eere.energy.gov/afdc/altfuel/biodiesel.html>). Biodiesel is the name given to these esters when they're intended for use as fuel. Approximately half of the biodiesel industry can use any fat or oil feedstock, including recycled cooking grease. The other half of the industry is limited to vegetable oils, the least expensive of which has been soy oil. The excess production capacity, product surpluses, and past prices of soy oil have been one of the key forces behind biodiesel commercialization. Current prices of soy oil have accelerated the industry's interest in utilization of alternative oil or fat sources for their initial feedstock.



There are presently 148 companies that account for the biodiesel sales of 250 million gallons even though their annual production capacity is 1.39 billion gallons. In addition, 96 companies report plants under construction and 5 plants are expanding within the next 18 months, which if realized, would result in an additional 1.89 billion gallons of biodiesel production. The principal co-product of the biodiesel production is crude glycerin (Ma and Hanna, 1999; van Gerpen, 2005), with 0.3 kg of crude glycerin generated for every gallon of biodiesel produced. As a result, one could expect approximately 75,000, 417,000, or 576,000 metric tons of crude glycerin generated from current sales, 2006 capacity, and expansion biodiesel estimates, respectively.

Glycerin has thousands of uses with new uses continuing to grow as new technologies are adapted. It is used to moisten, sweeten and preserve foods and drinks (soft drinks, candies, cakes, casings for meats and cheese, dry pet foods, etc.), widely used in drugs and pharmaceuticals (capsules, anesthetics, cough remedies, lozenges, emollient for skin medications, etc.), used as a moisturizing agent or emollient for cosmetics and toiletries (toothpaste, skin creams, deodorants, make up, lipstick, mascara, etc.), keeps tobacco moist and soft to prevent breaking and crumbling during processing (also adds flavor to chewing and pipe tobaccos, and used to manufacture cigarette filter tips), used to soften and reduce shrinkage during paper manufacturing (grease-proof paper, food wrappers, printing ink manufacturing, etc.), used to size and soften yarn and fabric, and produce a renewable propylene glycol (humectants, antifreeze and de-icing solutions, etc.). With the expansion in biodiesel production, the US crude glycerin market is inundated with an ample supply of product. In addition, increased global production of refined glycerin has pushed the price of refined glycerin from \$0.70 per pound to approximately \$0.30 per pound; consequently, crude glycerin which was once valued at \$0.25 per pound, is now closer to \$0.05 per pound. The 'silver lining' of a bountiful supply of inexpensive unrefined glycerin is that researchers are finding new uses for glycerin (glycerin and citric acid can be chemically combined to produce biodegradable polymers; biodegradable films, sheets, plastics, and gel-like coatings; propylene glycol; and E. coli conversion of glycerin into ethanol).

METABOLISM

During digestion, fats or oils are hydrolyzed by pancreatic lipase to form 2 free fatty acids and a 2-monoacylglyceride, all of which can be transported to the liver and ultimately broken down into glycerol and another free fatty acid in the liver (Mayes, 1985). Following digestion, intestinal absorption of glycerol has been shown to range from 70 to 90% in rats (Lin, 1977) to more than 97% in pigs and laying hens (Bartelt and Schneider, 2002). Glycerol is water soluble and can be absorbed by the stomach, but at a rate of absorption slower than that of the intestine (Lin, 1977). Absorption rates are high, likely due to its small molecular weight and it being passively absorbed rather than forming a micelle like that noted for medium and long chain fatty acids with bile salts (Guyton, 1991). Once absorbed, glycerol can be converted to glucose via gluconeogenesis or oxidized for energy production via glycolysis and citric acid cycle (Figure 3) which can account for 60% of the metabolic fate of glycerol under basal conditions (Robergs and Griffin, 1998). Glycerol metabolism largely occurs in the liver and kidney where the amount of glucose carbon arising from glycerol depends upon metabolic state and level of glycerol consumption (Lin, 1977; Hetenyi et al., 1983; Baba et al., 1995). With glycerol gluconeogenesis being limited by the availability of glycerol (Cryer and Bartley, 1973; Tao et al., 1983), crude glycerin has the potential of being a valuable dietary energy source for monogastrics.

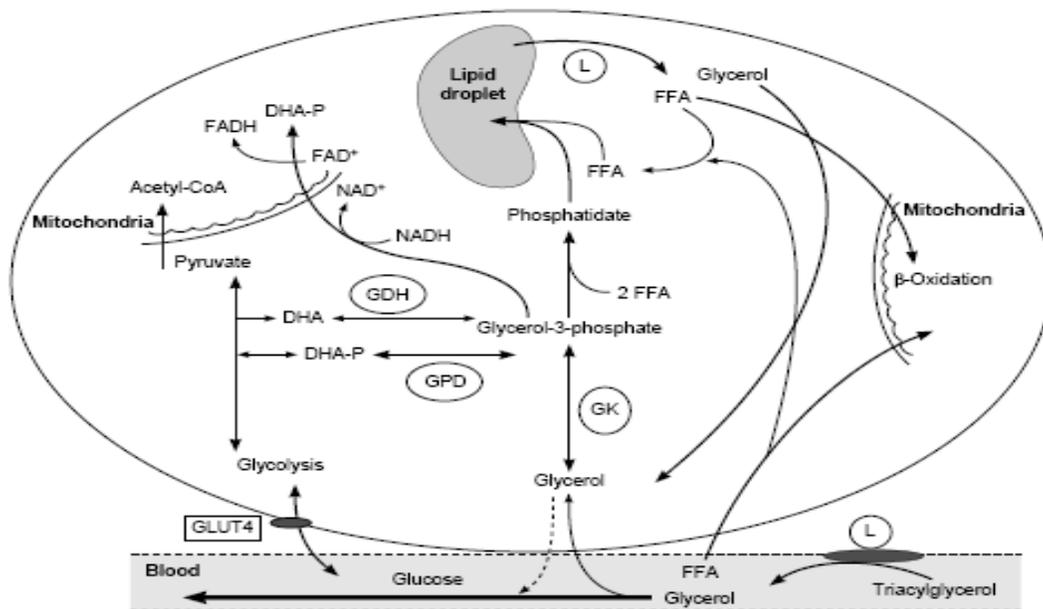


Fig. 3 Biochemical reactions involved in glycerol synthesis and metabolic conversion to glycerol-3-phosphate, phosphatidate and triacylglycerol. DHA = dihydroxyacetone; DHA-P = dihydroxyacetone phosphate; FAD⁺ = oxidised form of flavin adenine dinucleotide; FADH = reduced form of flavin adenine dinucleotide; FFA = free fatty acid; GDH = glycerol dehydrogenase; GK = glycerol kinase; GLUT4 = glucose transport protein; GPD = glycerol phosphate dehydrogenase; L = lipase; NAD⁺ = oxidised form of nicotinamide adenine dinucleotide; NADH = reduced form of nicotinamide adenine dinucleotide. **Robergs and Griffin, 1998**

EFFECTS ON PERFORMANCE, CARCASS COMPOSITION AND MEAT QUALITY

Schroder and Sudekum (1999) showed that dietary glycerin inclusions up to 20% had no effect on nutrient digestibilities in sheep. Glycerin delivered as a drench in high producing dairy cows has been shown to prevent ketoacidosis by increasing the supply of glucose precursors (Fisher et al., 1973; Sauer et al., 1973, Goff and Horst, 2001). In contrast, DeFrain et al. (2004) indicated that feeding glycerin-supplemented diets to transition dairy cows did not appear to exhibit the same glucogenic effect as noted when glycerin was drenched.

Several researchers have reported that glycerin is an acceptable feed ingredient for poultry (Campbell and Hill, 1962; Brambilla and Hill, 1966; Lin et al., 1976; Lessard et al., 1993; Simon et al., 1996; Cerrate et al., 2006). Adding glycerin up to an inclusion level of 5% has shown no adverse effects on growth or carcass yield (Lessard et al., 1993; Simon et al., 1996; Cerrate et al., 2006). However, increasing dietary glycerin above 10% has been shown to adversely affect growth performance and meat yield of broiler chickens (Simon et al., 1996; Cerrate et al., 2006), although this may be due to feed flowability (Cerrate et al., 2006). Rosebrough et al. (1980) found no adverse effects on egg production, egg weight, or feed utilization in turkey hens fed a pure source of glycerin as a source of energy over a 16-wk period.

German researchers (Kijora and Kupsch, 2006; Kijora et al., 1995, 1997) have suggested that up to 10% glycerin can be fed to pigs with little effect on pig performance. Likewise, Mourot et al. (1994) indicated that growth performance of pigs from 35 to 102 kg was not affected by the addition of 5% glycerin. The impact of dietary glycerin on carcass quality in pigs has been variable. Kijora et al. (1995) and Kijora and Kupsch (2006) showed no consistent effect of 5 or

10% glycerin addition on carcass composition or meat quality parameters, while in an additional study, pigs fed 10% glycerin exhibited slight increases in backfat, 45 min pH, flesh color, marbling, and leaf fat (Kijora et al., 1997). Although they did not note any change in saturated fatty acid profiles, there was a slight increase in oleic acid accompanied by a slight decrease in linoleic and linolenic acids resulting in an decline in the polyunsaturated to monounsaturated fatty acid ratio in backfat tissue. Likewise, Mourot et al. (1994) reported no consistent change in carcass characteristics due to 5% glycerin supplementation of the diet, but did note an increase in oleic acid and a reduction in linoleic acid in backfat and *semimembranosus* muscle tissue. Although Kijora and Kupsch (2006) did not note any effect of glycerin supplementation on meat dripping or press water loss, Mourot et al. (1994) reported a reduction in 24-h drip loss (average of 1.76 versus 2.27%) and cooking loss was also reduced (25.6 vs 29.4%) from the the *Longissimus dorsi* and *semimembranosus* muscles due to dietary supplementation with 5% glycerin.

GLYCERIN: METABOLIZABLE ENERGY DETERMINATION

Pure glycerin is a colorless, odorless, and a sweet-tasting viscous liquid, containing approximately 4.1 (Brambilla and Hill, 1966) to 4.3 (in-house analysis) kcal GE/g. Prior to initiation of our research, two samples of crude glycerin were analyzed to contain an average of 3625 kcal GE/kg, as-is. The reduced energy in crude glycerin compared with pure glycerin was not surprising, since the crude glycerin sample analyzed contained approximately 86% glycerin, 10% water, 3% NaCl, and a trace amount of free fatty acids. Previous data assumed the ME of glycerin as $\geq 95\%$ of its gross energy in dietary formulation (Brambilla and Hill, 1966; Lin et al., 1976; Rosebrough et al., 1980; Cerrate et al., 2006). However, data empirically determining the metabolizable energy content in crude glycerin is lacking.

In sheep, Schroder and Sudekum (1999) determined that glycerin contained between 1,982 and 2,316 kcal NE_L/kg. Recently, Bartlet and Schneider (2002) reported a decrease in the ME of glycerin as the level of dietary pure glycerin was increased in broiler, laying hen, and swine diets (Table 1). With the prececal digestibility of glycerin being $\geq 97\%$ (Bartlet and Schneider (2002), a possible explanation for this decrease in ME would be that blood glycerol levels may have increased (Kijora et al., 1995; Kijora and Kupsch, 1996; Simon et al., 1996) such that complete renal reabsorption was prevented, resulting in glycerol excretion in the urine (Kijora et al., 1995; Robergs and Griffin, 1998).

<u>Glycerin, %</u>	<u>Broiler, kcal/kg</u>	<u>Laying hen, kcal/kg</u>	<u>Swine, kcal/kg</u>
5	4,237	4,204	4,180
10	4,056	4,108	3,439
15	3,686	3,475	2,256

With the lack of data on the metabolizable energy content of crude glycerin, the following studies were conducted in broilers, laying hens, and swine. The sample of crude glycerin utilized for these studies was obtained from AGP Inc., Sergeant Bluff, IA, from a production facility using soy oil as its initial feedstock (Table 2).

<u>Specifications</u> ¹	<u>Value</u>	<u>Analytical method</u>
Total glycerin, %	86.95	ASTM D 6584-00E01
Methanol, %	0.028	Gas chromatography (proprietary method)
pH	5.33	Orion 230A pH meter with 9107 BN probe
Moisture, %	9.63	AOCS Ca 2e-84
NaCl, %	3.13	AOCS Db 7-48
Ash, %	3.19	AOCS Ca 11-55
Total fatty acid, %	0.29	AOCS G 4.40, modified for glycerin
<u>Analysis</u> ²	<u>Value</u>	<u>Analytical method</u>
Moisture, %	9.22	AOAC 984.20
Crude protein, %	0.41	AOAC 990.03
Crude fat, %	0.12	AOAC 920.39 (A)
Ash, %	3.19	AOAC 942.05
Sodium, %	1.26	AOAC 956.01
Chloride, %	1.86	AOAC 9.15.01, 943.01
Potassium, %	< 0.005	AOAC 956.01
Color	< 1	AOCS Cc 13a-43
Gross energy (kcal/kg) ³	3,625 ± 26	Adiabatic bomb calorimeter

¹Values reported by AGP Inc. Sergeant Bluff, IA, Lot # GB605-03.
²Analysis by University of Missouri-Columbia Experiment Station Chemical Laboratories, Columbia, MO.
³Analysis by USDA-ARS-SOMMRU, Ames IA (Model 1281, Parr Instrument Co. Inc., Moline, IL.)

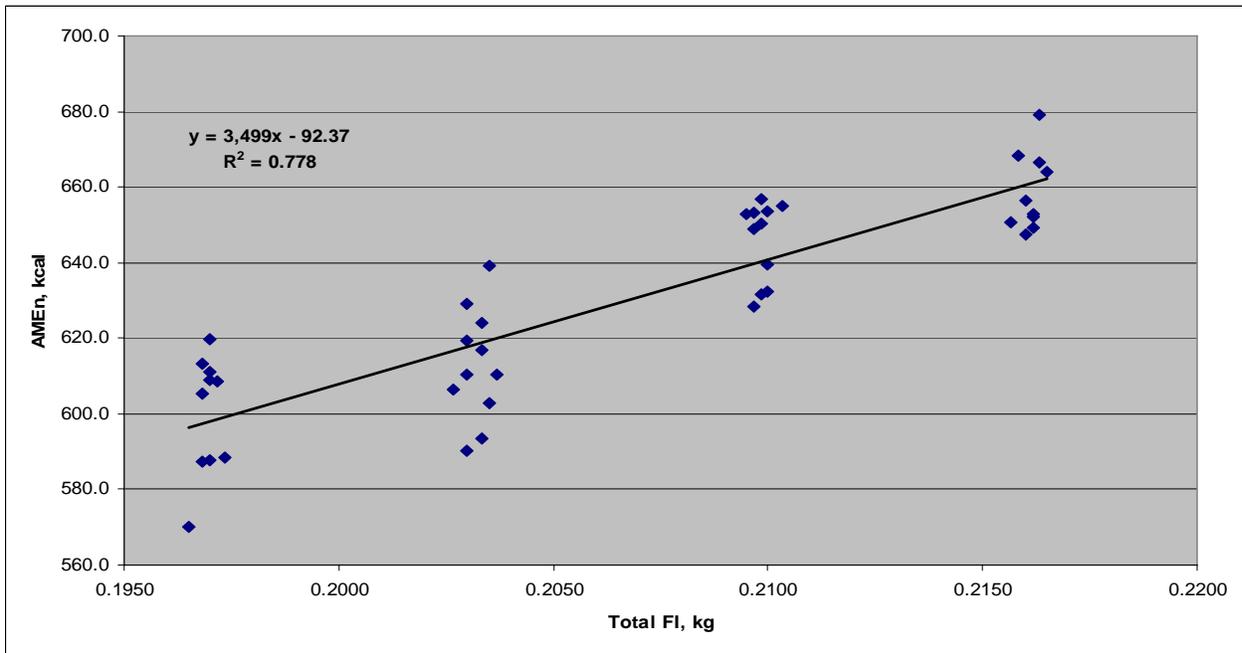
BROILER: USDA-ARS-PRU (Dozier et al., Poult. Sci.-submitted)

Materials and Methods: Three separate energy balance experiments (3 d acclimation followed by a 3 d collection period) were conducted using Ross × Ross 708 broilers obtained from a commercial hatchery that had been vaccinated at the hatchery for Marek’s disease, Newcastle disease, and infectious bronchitis. In all experiments, broilers were randomly distributed into grower battery cages 3 d prior to experimentation. In Exp. 1, 288 chicks were used from 4 to 11 d (12 chicks per cage; 6 males and 6 females), whereas 536 chicks from 17 to 25 d (12 chicks per cage; 6 males and 6 females) were used in Exp. 2 and 240 male broilers from 37 to 45 d (5 birds per cage) were used in Exp. 3. Basal diets were formulated to meet or exceed NRC (1994) nutrient recommendations for broiler chickens used in each experiment except that all diets were low in AME_n because no dietary fat was added. Experimental diets were created by the addition of crude glycerin (as characterized in Table 2) to the basal diet. In Exp. 1, two dietary treatments were formulated, consisting of a control diet (100% basal diet) and a diet containing 6% glycerin (94% basal diet + 6% glycerin). In Exp. 2 and 3, dietary treatments were the addition of glycerin at 0 (100% basal diet), 3% (97% basal diet + 3% glycerin), 6% (94% basal diet + 6% glycerin), and 9% (91% basal diet + 9% glycerin). Experiment 1 was a preliminary study that estimated AME_n by difference, where AME_n of the control diet was subtracted from the AME_n of the diet containing 6% glycerin. In Exp. 2 and 3, broilers were fed 91, 94, 97, and 100% of ad libitum intake which allowed for each treatment group to consume the same amount of basal diet so

differences in AME_n were due to glycerin. Subsequently, AME_n intake was regressed against feed intake with the slope representing AME_n of glycerin (Adeola, 2001).

Results: In Exp. 1, with broiler chicks from 7 to 10 d of age, AME_n of glycerin was determined as 3,877 kcal/kg. In Exp 2, AME_n of glycerin was estimated as 3,499 kcal/kg utilizing 21 to 24 d old broilers [Figure 4: $Y = 3,499x - 92.37$ ($P \leq 0.0001$; $r^2 = 0.778$)]. In Exp 3, glycerin was estimated to contain 3,675 kcal AME_n/kg with 42 to 45 d old broilers [Figure 5: $Y = 3,675x - 226.53$ ($P \leq 0.0001$; $r^2 = 0.844$)]. No quadratic or cubic effects were noted.

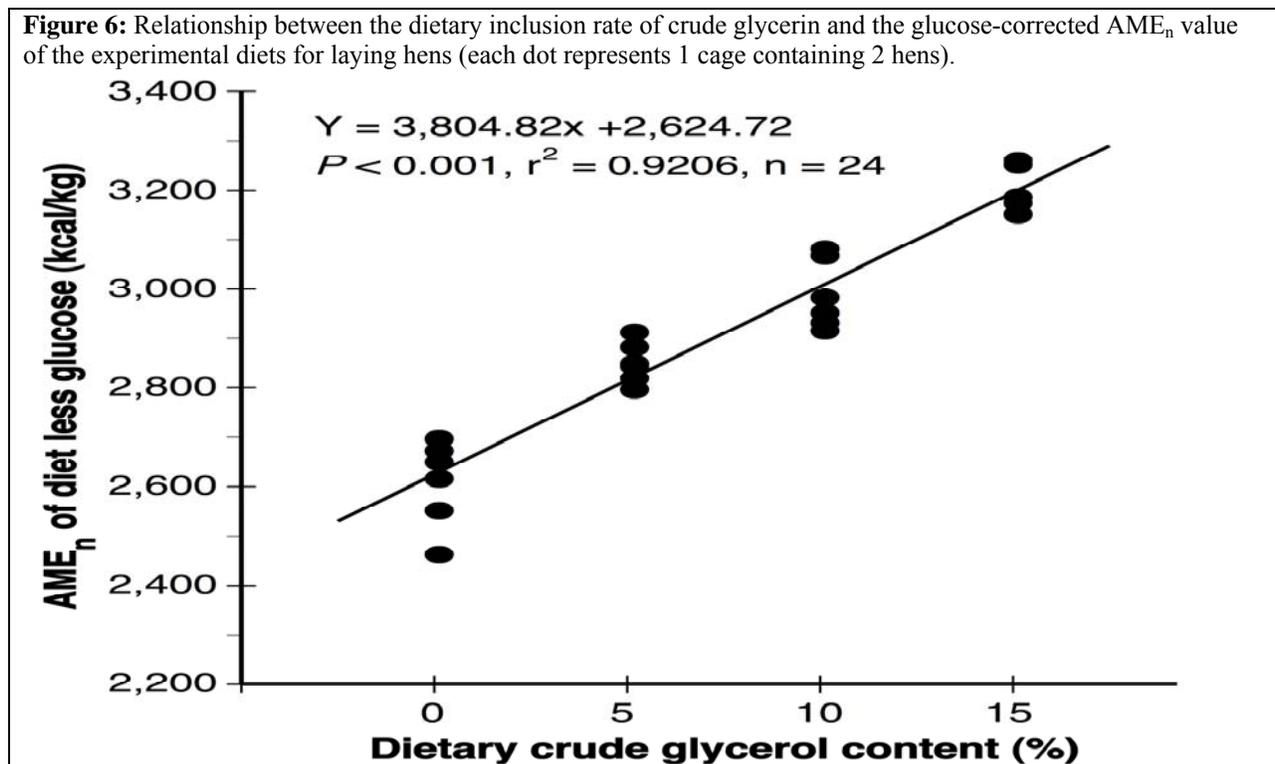
Figure 4. Regression of AME_n intake vs. feed intake from 21 to 24 d of age in Exp. 2.



Materials and Methods: Forty-eight, 40-wk-old Single Comb White Leghorn laying hens (Hy-Line W-36) were obtained from a commercial source and placed, 2 per cage, into metabolic cages. Upon arrival, the hens were given free access to a standard laying hen diet and water for a 2-wk acclimatization period, after which each cage was randomly assigned to 1 of the 4 experimental diets. Hens were given free access to the experimental diets for a 7-d adaptation period followed by a 3-d collection period. Four experimental diets were used, formulated from a basal diet to which 5, 10, or 15% crude glycerin (as characterized in Table 2) was substituted for glucose•H₂O on an equal weight basis (Sell et al., 2001). All diets were formulated to meet or exceed the NRC (1994) nutrient recommendations and contained 1.0% Celite as an indigestible marker. Egg production was recorded daily and the feed consumption was recorded for the 10-d-long experiment. Eggs collected on d 7 and 8 of the experiment were weighed and egg mass was calculated. Excreta was collected twice daily on d-8 through 10 and stored at -20°C until analysis. The AME_n content of the crude glycerin was estimated by a linear regression equation relating the experimental diet AME_n values to proportion of crude glycerin in each diet (Leeson and Summers, 2001; Sell et al., 2001).

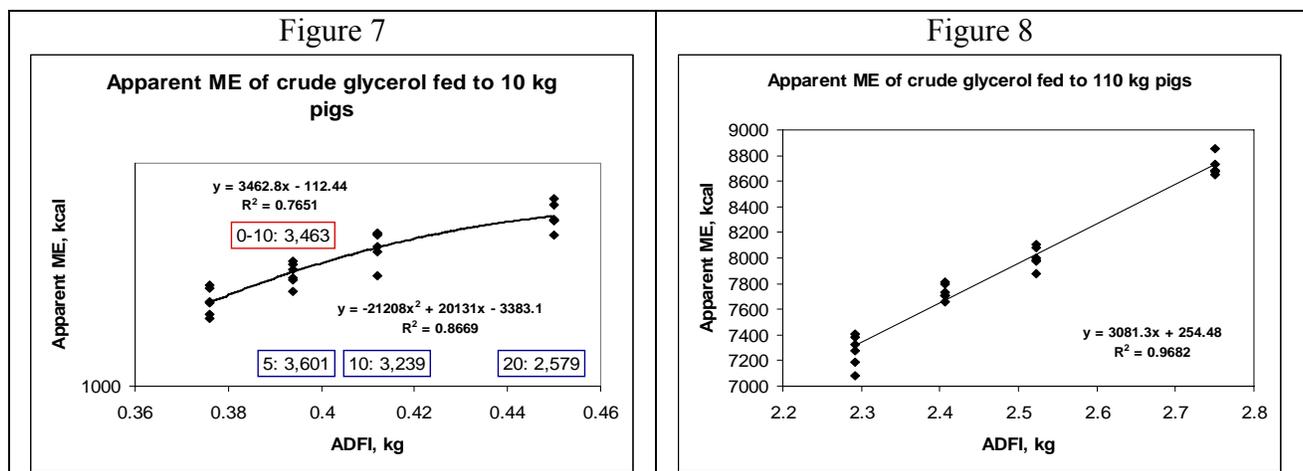
Results: Mean BW of the hens was 1.37 kg at the start of the experiment with no difference among treatments. The AME_n value of the crude glycerin tested was 3,805 ± 238 kcal/kg (linear, $P < 0.001$) with no quadratic or cubic effects, (Figure 6). Feed consumption, egg production, egg weight, or egg mass were not affected by substitution of crude glycerin for glucose•H₂O during the 7 d adaptation period, the 3 d collection period, or the entire 10 d experiment.

Figure 6: Relationship between the dietary inclusion rate of crude glycerin and the glucose-corrected AME_n value of the experimental diets for laying hens (each dot represents 1 cage containing 2 hens).



Materials and Methods: Three experiments (Exp. 1, 3, and 4) examined crude glycerin fed to starter pigs (average initial BW=10.3 kg) while two experiments (Exp. 2 and 5) examined crude glycerin fed to finishing pigs (average initial BW=104.7 kg). In each experiment, 24 pigs were randomly assigned to individual metabolism crates equipped with screens and trays that allowed total but separate collection of feces and urine. Due to crate design, barrows were used in the starter pig metabolism experiments while gilts were used in the finishing pig metabolism experiments. Dietary treatments consisted of a common basal diet which met or exceeded the NRC (1988) requirements mixed with 0, 5, 10, or 20% crude glycerin (Exp. 1 and 2) or 0 and 10% crude glycerin (Exp. 3, 4, and 5). A 10 d adjustment period was used to adapt pigs to the metabolism crate and the dietary treatment; and to determine the appropriate meal size when fed twice daily. In Exp. 1 and 2, pigs were fed a set amount of the basal diet with pigs on the glycerin treatments offered an increased feed allotment based upon the amount of glycerin addition to the basal mix (Adeola, 2001). In Exp. 3, 4, and 5, all pigs used in a specific experiment were offered the same amount of feed, regardless of glycerin addition. The apparent DE and ME values of crude glycerin fed to pigs were estimated as the slope of the linear relationship between the apparent DE and ME value of the experimental diet (dependent variable) and feed intake (independent variable).

Results: The GE of crude glycerin evaluated in these experiments (3,625 kcal/kg) was close to expectations relative to pure glycerin (in-house GE analysis of 4,305 kcal/kg), given that our sample of crude glycerin evaluated contained 86.95% glycerin with low levels of methanol (0.028%) and free fatty acid (0.29%). Based upon our data in broilers and laying hens we did not expect the level of crude glycerin to affect ME determination. However, when data from Exp. 1 was analyzed separately, the ME of crude glycerin declined with increasing levels of supplementation, with estimated ME values of 3,601, 3,239, and 2,579 kcal/kg crude glycerin for 5, 10, and 20% inclusion levels, respectively (Figure 7, quadratic, $P = 0.05$). In Exp. 1, the decrease in ME of glycerin appears to be due to pigs fed the 20% crude glycerin. Omitting the 20% inclusion level data from Exp. 1 showed no such difference in ME estimation with the remaining levels of crude glycerin tested (0, 5, and 10%), resulting in a ME value of 3,463 kcal/kg (Figure 7, linear, $P = 0.0001$). In contrast, there was no effect of crude glycerin inclusion level on ME determination when determined in finishing pigs, Exp. 2, Figure 8.

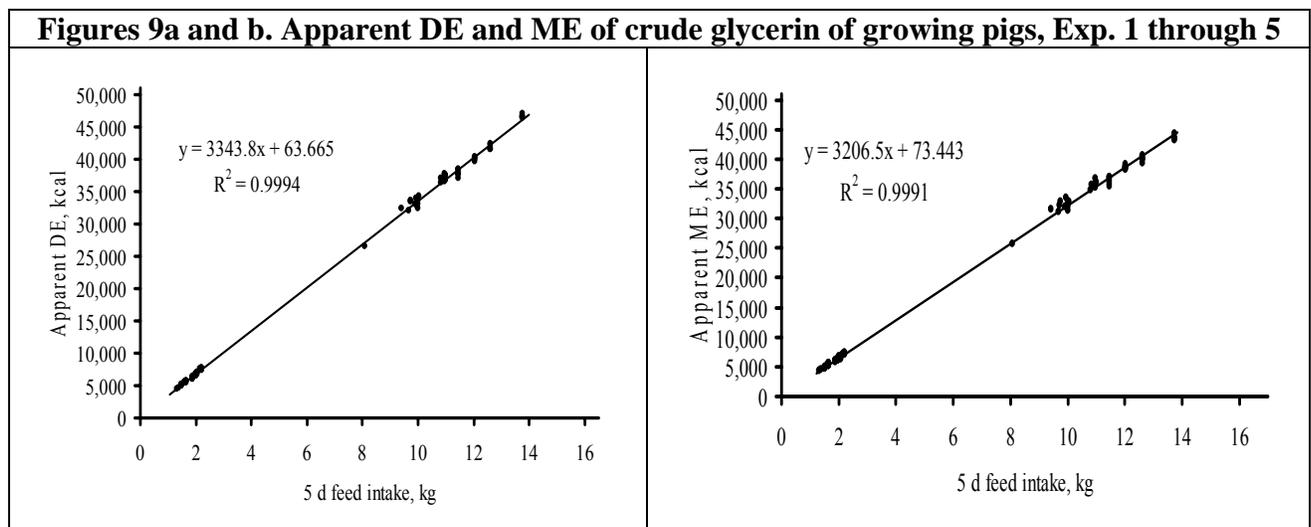


Because pigs fed the 20% crude glycerin in Exp. 1 had reduced utilization of crude glycerin, as determined by a lower ME estimate, we chose to exclude these pigs from subsequent analysis. Table 3 represents the apparent DE and ME values as determined by linear regression for Exp. 1 to 5, respectively. Apparent DE and ME were not influenced by experiment (Exp. 1 to 5), method of fecal collection time points (Exp. 1 and 2 versus Exp. 3, 4, and 5), or by type of pig (starter-Exp. 1, 3, and 4 versus finisher-Exp. 2 and 5).

<u>Experiment</u>	<u>Pigs</u>	<u>Initial BW, kg</u>	<u>DE, kcal/kg</u>	<u>SEM</u>	<u>ME, kcal/kg</u>	<u>SEM</u>
1 ²	18	11.0±0.6	4,401	282	3,463	480
2 ³	23	109.6±5.5	3,772	108	3,088	118
3 ⁴	19	8.4±0.9	3,634	218	3,177	251
4 ⁴	20	11.3±0.7	4,040	222	3,544	237
5 ⁴	22	99.9±7.4	3,553	172	3,352	192

¹ All experiments represent data from 5 d energy balance experiments following a 10 d adaptation period.
² Included pigs fed 0, 5, and 10% crude glycerin.
³ Included pigs fed 0, 5, 10, and 20% crude glycerin.
⁴ Included pigs fed 0 and 10% glycerin.

In these experiments, the ratio of DE:GE equaled 92% suggesting that crude glycerin was well digested by pigs and is supported by Bartlet and Schneider (2002) who reported that >97% of the glycerin is digested prior to the cecum. The ratio of ME:DE for the crude glycerin examined was 96% which is identical to the ME:DE ratio for soybean oil, and is comparable to the ratio of ME:DE for corn grain which is 97% (NRC, 1998). Combining all experiments show that the DE value of crude glycerin (86.95%) used was 3,344 kcal/kg (Figure 9a) while the ME was 3,207 kcal/kg (Figure 9b). When placed on an equivalent glycerin basis, this ME determination would be slightly higher than the 3,436 kcal ME/kg determined for pure glycerin by Bartlet and Schneider (2002).



Materials and Methods: Eight days post-weaning, 96 pigs (48 gilts, 48 barrows, 8.0 kg BW) were allotted to 24 pens with 4 pigs per pen. Dietary treatments were 0, 5, and 10% crude glycerin formulated to be equal in energy and digestible lysine. The composition of crude glycerin was: 84.51% glycerin, 12.24% water, 2.93% NaCl, and 0.318% methanol. Pigs were fed for 138 d in 5 growth phases, with all pens changed to the next phase at the same time. On d-138, all pigs (average BW 133.2 kg) and feeders were weighed for performance calculations and individual pigs scanned using real-time ultrasound for estimates of fat depth and loin muscle area. The following day, all pigs were shipped to a commercial packer for tissue harvesting.

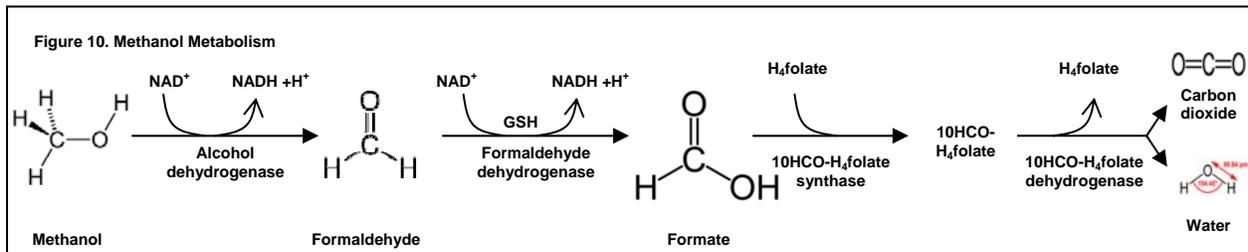
Results: Dietary glycerin had no effect on ADG, ADFI, or GF from d-0 to 19 or from d-0 to 138. Glycerin supplementation did not affect backfat depth, longissimus area, or percentage fat free lean (Table 4). Longissimus samples are being evaluated for meat quality indices and lipid fatty acid composition; plasma metabolites; and pathology of eye, liver, and kidney samples.

Criterion	Crude glycerin, %			SEM	P value
	0	5	10		
<u>d 0 to 19¹</u>					
ADG, g/d	353	360	351	15.2	0.90
ADFI, g/d	506	530	504	15.9	0.44
G:F	0.699	0.678	0.695	0.019	0.69
<u>d 0 to 138</u>					
ADG, g/d	905	913	906	15.7	0.93
ADFI, g/d	2,333	2,385	2,400	52.4	0.65
G:F	0.386	0.382	0.376	0.003	0.13
scanBF, mm	18.8	21.0	20.7	0.8	0.14
scanLEA, cm ²	48.6	49.0	46.6	0.9	0.12
Scan %FFL, carcass	52.9	52.5	51.7	0.4	0.14
¹ Eight pens per treatment, 4 pigs per pen, 8.0 to 14.7 kg BW, d 0 to 19.					
² 8.0 to 133.2 kg, d-0 to 138.					

GLYCERIN: QUALITY VARIATIONS

Biodiesel can be produced by a variety of feedstocks, such as oils from soy, canola, and corn, waste cooking oils, and animal fats; and due to the biodiesel production process (Ma and Hanna, 1999; Van Gerpen, 2005; Thompson and He, 2006). Consequently, the composition of crude glycerin can vary, but ranges from: 78 to 85% glycerin, 8 to 15% water, 2 to 10% salt (NaCl or KCl), 0.5% free fatty acids (although non-acidulated products may be up to 25% FFA), and ≤ 0.5% methanol. Given these general specifications, the amount of salt and methanol arise as formulation concerns. Depending upon the level of salt, inclusion levels may need to be limited depending upon the species being fed. However, data suggests that in swine and poultry, up to 3% dietary NaCl will have no adverse effects in animal performance, (adapted from NRC, 1980), although the impact of increased water intake on manure composition (Sutton et al., 1976) or wet litter (Hogge et al., 1999) would need to be considered.

Methanol levels in crude glycerin warrant special consideration. Methanol is a potentially toxic compound and has been reviewed in detail by others (Roe, 1982; Medinsky and Dorman, 1995; Skrzydlewska, 2003). Methanol can be introduced orally, by respiration, or through the skin, and is distributed by the blood to all organs and tissues in proportion to their water content (Liesivuori and Savolainen, 1991). Metabolic elimination of methanol is much slower than that of ethanol, with its metabolism shown in Figure 10.



Small amounts of methanol are directly excreted in the kidney and lung, but the majority is metabolized by the liver and released as CO₂. Acute methanol intoxication is initially manifested by signs of narcosis followed by a latent period in which formic acid accumulates causing metabolic acidosis (reduced blood pH, depletion of blood bicarbonate, visual degeneration, and abdominal, leg, and back pain). Chronic exposure causes headache, insomnia, gastrointestinal problems, and blindness. Animals differ widely in their ability to metabolize methanol depending upon enzyme activity and hepatic folate levels (Roe, 1982; Black et al., 1985; Medinsky and Dorman, 1995; Skrzydlewska, 2003). Little research on methanol metabolism or toxicity has been conducted in the pig. Makar et al. (1990) reported that the pig, compared to all other species studies, has extremely low levels of folates and very low level of a key enzyme in the folate pathway, 10-formyl H₄folate dehydrogenase, suggesting the ability of the pig to dispose of formate was limited and slower than that observed in rats or monkeys. Dorman et al. (1993), however, indicated that methanol- and formate-dosed minipigs did not develop optic nerve lesions, toxicologically significant formate accumulation, or metabolic acidosis, indicating that female minipigs do not appear to be overtly sensitive to methanol toxicity. With the concern with potential methanol and formate toxicity, it is interesting to note that formaldehyde (CFR573.460) can be used as a silage preservative and formic acid (CFR 573.640) can be used in finished feeds to reduce bacterial loads. Formic acid or formate salts have been safely used in swine (Overland et al., 2000; Canibe et al., 2005) and formaldehyde in laying hens (Khan et al., 2006). It is also interesting to note that calcium formate can be used as a dietary calcium supplement in humans (Hanzlik et al., 2005).

Pure glycerin is regulated under CFR582.1320 as a substance that is GRAS for general purpose use in animal feed when used in accordance with good manufacturing or feeding practices. With no GRAS regulation or AAFCO definition listing specifications for crude glycerin use in animal feeds, specifications for pure glycerin defined under United States Pharmacopeia (USP) and Food and Chemical Codex (FCC) specifications are used for guidance. Methanol levels, however, are not specifically listed in the USP or FCC specifications, such that the FDA has decided to address free methanol levels under CFR573.640, regulation 21, requiring that levels of methanol in methyl esters of higher fatty acids should not exceed 150 ppm (0.015%) or a level shown to be safe for use in animal diets. In Europe, German regulations allow 0.5% (500 ppm)

methanol in crude glycerin (Normenkommission für Einzelfuttermittel im Zentralkomitee der Deutschen Landwirtschaft. 2006).

CONCLUSIONS

With an AME_n value of the crude glycerin sample tested determined to be 3,684 and 3,805 kcal/kg in broilers and laying hens, respectively, and 3,207 kcal ME/kg in swine, crude glycerin can be used as an excellent source of calories in nonruminants. As reported by others as well as our current research in growing-finishing pigs, levels of crude glycerin up to 10% appear to have little impact on pig performance, carcass composition, or meat quality. Levels of other compounds in crude glycerin (i.e., methanol, sodium- or potassium chloride, and free fatty acids), however, must be monitored for potential impacts on ME determination and on performance responses to this feedstuff. In addition, impacts on feed handling and manufacturing characteristics need to be considered on the level of crude glycerin that can be supplemented in diets fed to poultry and swine.

LITERATURE CITED

- Adeola, O. 2001. Digestion and balance techniques in pigs. Pages 903-916 in Swine Nutrition. A. J. Lewis and L. L. Southern., 2nd ed. CRC Press, New York.
- Alternative Fuels Data Center. 2007. <http://www.eere.energy.gov/afdc/altfuel/biodiesel.html>, Accessed July 2007.
- American Oil Chemists' Society. 2000. Official Methods and Recommended Practices of the American Oil Chemists' Society. 5th ed. American Oil Chemists' Society, Champaign, IL.
- American Society for Testing and Materials Standards International. 2006. Annual Book of American Society for Testing and Materials Standards International, Vol. 05.04, Petroleum Products and Lubricants (IV): D6557–Latest, ASTM International, West Conshohocken, PA.
- Annual Energy Outlook. 2007. <http://www.eia.doe.gov/oifa/aeo/>, Accessed July 2007.
- Baba, H., X.-J. Zhang, and R. R. Wolfe. 1995. Glycerol gluconeogenesis in fasting humans. *Nutr.* 11:149-153.
- Bartlet, J., and D. Schneider. 2002. Investigation on the energy value of glycerol in the feeding of poultry and pig. Pages 15-36 in Union for the Promotion of Oilseeds-Schriften Heft 17.
- Black, K. A., J. T. Eells, P. E. Neker, C. A. Hawtrey, and T. R. Tephly. 1985. Role of hepatic tetrahydrofolate in the species difference in methanol toxicity. *Proc. Natl. Acad. Sci.* 82:3854-3858.
- Brambilla, S., and F. W. Hill. 1966. Comparison of neutral fat and free fatty acids in high lipid-low carbohydrates diets for the growing chicken. *J. Nutr.* 88:84-92.
- Campbell, A. J., and F. W. Hill. 1962. The effects of protein source on the growth promoting action of soybean oil, and the effect of glycerine in a low fat diet. *Poult. Sci.* 41:881-882.
- Canibe, N., O. Hojberg, S. Hojsgaard, and B. B. Jensen. 2005. Feed physical form and formic acid addition to the feed affect the gastrointestinal ecology and growth performance of growing pigs. *J. Anim. Sci.* 83:1287-1302.
- Cerrate, S., F. Yan, Z. Wang, C. Coto, P. Sacakli, and P. W. Waldroup. 2006. Evaluation of glycerine from biodiesel production as a feed ingredient for broilers. *Int. J. Poult. Sci.* 11:1001-1007.

- Cryer, A., and W. Bartley. 1973. Studies on the adaptation of rats to a diet high in glycerol. *Int. J. Biochem.* 4:293-308.
- DeFrain, J. M., A. R. Hippen, K. F. Kalscheur, and P. W. Jardon. 2004. Feeding glycerol to transition dairy cows: effects on blood metabolites and lactation performance. *J. Dairy Sci.* 87: 4195-4206.
- Dorman, D. C., J. A. Dye, M. P. Nassise, J. Ekuta, B. Bolon, and M. A. Medinsky. 1993. Acute methanol toxicity in minipigs. *Fund. Appl. Toxicol.* 20:341-347.
- Fisher, L. J., J. D. Erfle, G. A. Lodge, and F. D. Sauer. 1973. Effects of propylene glycol or glycerol supplementation of the diet of dairy cows on feed intake, milk yield and composition, and incidence of ketosis. *Can. J. Anim. Sci.* 53: 289-296.
- Goff, J. P., and R. L. Horst. 2001. Oral glycerol as an aid in the treatment of ketosis/fatty liver complex. *J. Dairy Sci.* 84(Suppl. 1):153. (Abstr.).
- Guyton, A. C. 1991. *Textbook of Medical Physiology*. W. B. Saunders Co., Philadelphia, PA.
- Hanzlik, R. P., S. C. Fowler, and J. T. Eells. 2005. Absorption and elimination of formate following oral administration of calcium formate in female human subjects. *Drug Metab. Disp.* 23:282-286.
- Hetenyi, G., G. Perez, and M. Vranic. 1983. Turnover and precursor-product relationships of nonlipid metabolites. *Physiol. Rev.* 63:606-667.
- Hill, J., E. Nelson, D. Tilman, S. Polasky, and D. Tiffany. 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. *PNAS* 103:11206-11210.
- Hogge, D. M., K. R. Kummings, and J. L. McNaughton. 1999. Evaluation of sodium bicarbonate, chloride, or sulfate with a coccidiostat in corn-soy or corn-soy-meat diets for broiler chickens. *Poult. Sci.* 78:1300-1306.
- Iowa Corn Promotion Board. 2007. <http://www.iowacorn.org/>, Accessed July 2007.
- Khan, A., S. M. Hussain, and M. Z. Khan. 2006. Effects of formalin feeding or administering into the crops of white leghorn cockerels on hematological and biochemical parameters. *Poult. Sci.* 85:1513-1519.
- Kijora, C., and S.-D. Kupsch. 2006. Evaluation of technical glycerols from "biodiesel" production as a feed component in fattening of pigs. *Lipid-Fett* 98:7:240-245.
- Kijora, C., R.-D. Kupsch, H. Bergner, C. Wenk, and A. L. Prabucki. 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. *J. Anim. Physiol. Anim. Nutr.* 77:127-138.
- Kijora, C., H. Bergner, R.-D. Kupsch, and L. Hageman. 1995. Glycerol as feed component in diets of fattening pigs. *Arch. Anim. Nutr.* 47:345-360.
- Leeson, S., and J. Summers. 2001. *Nutrition of the chicken*. 4th ed. University Books, Guelph, ON.
- Lessard, P., M. R. Lefrancois, and J. F. Bernier. 1993. Dietary addition of cellular metabolic intermediates and carcass fat deposition in broilers. *Poult. Sci.* 72:535-545.
- Liesivuori, J., and H. Savolainen. 1991. Methanol and formic acid toxicity: biochemical mechanisms. *Pharmacol. Toxicol.* 69: 157-163
- Lin, M. H., D. R. Romsos, and G. A. Leveille. 1976. Effect glycerol on enzyme activities and on fatty acid synthesis in the rat and chicken. *J. Nutr.* 106:1668-1677.
- Lin, E. C. C. 1977. Glycerol utilization and its regulation in mammals. *Annu. Rev. Biochem.* 46:765-795.
- Ma, F., and M. A. Hanna. 1999. Biodiesel production: A review. *Biores. Tech.* 70:1-15.

- Makar, A. B., T. R. Tephly, G. Sahin, and G. Osweiler. 1990. formate metabolism in young swine. *Toxicol. Appl. Pharm.* 105:315-320.
- Mayes, P. A. 1985. Metabolism of carbohydrate. Harper's Review of Biochemistry, 20th ed., Martin, Mayes, Rodwell, and Granner, 720 pp, Lange Medical Publications, Los Altos, CA.
- Medinsky, M. A., and D. C. Dorman. 1995. Recent developments in methanol toxicity. *Toxicol. Letters* 82/83:707-711.
- Mourot, J., A. Aumaitre, A. Mounier, P. Peiniau, and A. C. Fracois. 1994. Nutritional and physiological effects of dietary glycerol in the growing pig. Consequences on fatty tissues and post mortem muscular parameters. *Livest. Prod. Sci.* 38:237-244.
- National Biodiesel Board. 2007. <http://www.biodiesel.org/>, Accessed May 2007.
- Normenkommission fur Einzelfuttermittel im Zentralausschuss der Deutschen Landwirtschaft. 2006. Positivliste fur Einzelfuttermittel, 5. Auflage, #12.07.03, p. 35.
- NRC. 1998. Nutrient requirements of swine. 10th rev. Ed. Natl. Acad. Press, Washington, DC.
- NRC. 1994. Nutrient requirements of poultry. 9th ed. Natl. Acad. Press, Washington, DC.
- NRC. 1980. Mineral tolerance of domestic animals. Natl. Acad. Press, Washington, DC.
- Overland, M., T. Granli, N. P. Kjos, O. Fjetland, S. H. Steien, and M. Stokstad. 2000. Effect of dietary formats on growth performance, carcass traits, sensory quality, intestinal microflora, and stomach alterations in growing-finishing pigs. *J. Anim. Sci.* 78:1875-1884.
- Robergs, R. A., and S. E. Griffin. 1998. Glycerol: biochemistry, pharmacokinetics and clinical and practical applications. *Sports Med.* 26:145-167.
- Roe, Oluf. 1982. Species differences in methanol poisoning. *CRC Critical Reviews in Toxicology* 10:275-286.
- Rosebrough, R. W., E. Geis, P. James, H. Ota, and J. Whitehead. 1980. Effects of dietary energy substitutions on reproductive performance, feed efficiency, and lipogenic enzyme activity on large white turkey hens. *Poult. Sci.* 59:1485-1492.
- Sauer, F. D., J. D. Erfle, and L. J. Fisher. 1973. Propylene glycol and glycerol as a feed additive for lactating dairy cows: an evaluation of blood metabolite parameters. *Can. J. Anim. Sci.* 53: 265-271.
- Schroder, A., and H. H. Sudekum. 1999. Glycerol as a by-product of biodiesel production in diets for ruminants. 10th International Rapeseed Congress, Canberra, Australia, Paper No. 241.
- Sell, J. L., S. Jin, and M. Jeffrey. 2001. Metabolizable energy value of conjugated linoleic acid for broiler chicks and laying hens. *Poult. Sci.* 80:209-214.
- Simon, A., H. Bergner, and M. Schwabe. 1996. Glycerol-feed ingredient for broiler chickens. *Arch. Anim. Nutr.* 49:103-112.
- Skrzydowska, E. 2003. Toxicological and metabolic consequences of methanol poisoning. *Toxicol. Mechanisms Methods* 13:277-293.
- Sutton, A. L., V. B. Mayrose, J. C. Nye, and D. W. Nelson. 1976. Effect of dietary salt level and liquid handling systems on swine waste composition. *J. Anim. Sci.* 43:1129-1134
- Tao, R. C., R. E. Kelley, N. N. Yoshimura, and F. Benjamin. 1983. Glycerol: Its metabolism and use as an intravenous energy source. *J. Parenteral Enteral. Nutr.* 7:479-488.
- Thompson, J. C., and B. B. He. 2006. Characterization of crude glycerol from biodiesel production from multiple feedstocks. *Appl. Eng. Agric.* 22:261-265.
- Van Gerpen, J. 2005. Biodiesel processing and production. *J. Fuel Proc.* 86:1097-1107.