

Abstract

SHIELDS, MICHAEL CHRISTOPHER. Evaluation of the Nutritional Value of Glycerol, a Byproduct of Biodiesel Production, for Swine. (Under the direction of Eric van Heugten.)

The objectives of this study were to: 1) evaluate diet manufacturing characteristics and feed mill processing data when using glycerol, 2) determine the value of glycerol in diets for newly weaned pigs relative to lactose, and 3) determine the value of glycerol in diets for nursery pigs when replacing lactose and corn.

For the first objective, two experiments were performed to test the performance of glycerol in the feed mill. In Experiment 1, finisher feed was mixed in a 50 kg mixer at glycerol levels of 0, 2.5, and 5%. Diets were manufactured in batches of 100 lbs in a randomized block design and this was replicated three times. In Experiment 2, finisher feed was mixed and pelleted to examine feed mill performance at glycerol levels of 0, 2.5, and 5%. Diets were manufactured in batches of 1,000 lbs in a randomized block design and this was replicated three times. In Experiment 1, as glycerol levels increased (0, 2.5 and 5%) flowability increased ($P = 0.03$) linearly (26.53, 25.2, 23.73 mm disc size respectively). In Experiment 2, pellet mill efficiency linearly increased ($P = 0.01$) by 27% and pellet durability were linearly increased ($P = 0.004$) by 46% as glycerol levels increased. Hot pellet temperature decreased ($P = 0.05$) by 2% and delta temperature decreased ($P = 0.02$) by 30% as glycerol levels increased.

For the second objective, a performance study was conducted. A total of 126 pigs (body weight was 6.68 ± 0.17 kg) were weaned at approximately 21 days of age, blocked by weight, and allocated to 42 pens with 3 pigs per pen. Pens were randomly assigned one of

six treatments in a 2x3 factorial randomized complete block design with factors: 1) glycerol inclusion in phase 1 diets (0 or 5%), and 2) glycerol inclusion level in phase 2 diets (0, 5, or 10%). Phase 1 diets were fed for 2 weeks and glycerol was supplemented to replace lactose on a weight-for-weight basis. Phase 2 diets were fed for 3 weeks and glycerol was included in replacement of corn and this replacement was made on a nutrient basis (thus accounting for the nutrient composition of corn). Results showed that replacing corn at levels of up to 10% improved ADG ($P < 0.002$) by 16%, ADFI ($P < 0.003$) by 21%, and G:F ($P < 0.044$) by 4%. Serum glycerol concentration was not impacted by the glycerol supplementation during the starter 1 phase, but a 16% linear increase in serum glycerol concentration during the starter 2 phase ($P < 0.0001$) was observed as dietary glycerol increased. Glycerol supplementation had no effect on serum glucose, total protein, albumin, bilirubin, creatine phosphokinase, globulin, and aspartate aminotransferase in the starter 1 phase. In the starter 2 phase, glycerol supplementation increased urea nitrogen ($P = 0.002$), decreased creatinine ($P = 0.02$), and increased the ratio of blood urea nitrogen to creatinine ($P = 0.0002$). Glycerol supplementation in the starter 2 phase had a quadratic effect on urea nitrogen ($P = 0.0008$), which was lower at 5% level of glycerol (4.71 mg/dl) compared to the 0 and 10% (6.86 and 7.43 mg/dl respectively). Cholesterol concentration was higher for 5% added glycerol (92.21 mg/dl) than for the 0% and 10% levels (79.14 and 84.64 mg/dl respectively; quadratic effect, $P = 0.04$). Glycerol supplementation in the starter 2 phase linearly decreased creatinine concentration ($P = 0.02$).

For the third objective, a performance trial was completed. A total of 144 pigs (body weight was 6.68 ± 0.17 kg) were weaned at approximately 21 days of age, blocked by

weight, and allocated to 28 pens with 3 pigs per pen. Pens were randomly assigned one of six dietary treatments: (0, 2.5, 5, 7.5, 10% glycerol added to replace 10% lactose in a basal starter 1 diet (fed for two weeks) containing 20% total lactose, and a negative control with 10% lactose and 0% glycerol). A common starter diet was fed for the remaining two weeks. Pigs were weighed and feed intake was measured weekly. Results demonstrated that glycerol supplementation at 10% compared to the negative control resulted in a greater ADG during weeks 1, 2, and the starter 1 period ($P = 0.03$) with ADG increased in pigs fed 10% glycerol increased by 30%. Glycerol also improved feed efficiency in the starter 1 period ($P < 0.04$). There was no impact of feeding glycerol in the starter 1 phase on subsequent performance during the starter 2 phase. Serum glycerol was linearly increased (7.1, 7.5, 31.1, 128.2, and 97.0 mg/dl) ($P = 0.03$) as glycerol levels increased. Glycerol supplementation in starter 1 diets had no effect on glucose, urea nitrogen, total protein, albumin, alkaline phosphatase, aspartate amino transferase, alanine amino transferase, cholesterol, calcium, phosphorus, sodium, potassium, chloride, albumin, globulin, the ratio of blood urea nitrogen to creatinine (buncreatinine), and creatine phosphokinase. Glycerol supplementation decreased creatinine ($P=0.0004$) and bilirubin ($P=0.02$) as dietary glycerol levels increased.

Overall these studies indicate that glycerol can be an asset in feedmilling by improving pellet durability, flowability, pellet mill efficiency, and hot pellet temperature. In nursery diets, it improves ADG, and ADFI at levels up to 10%. Overall data indicated that glycerol can be added in the diets at levels up to 5% and improve feedmill performance and pig performance.

Evaluation of the Nutritional Value of Glycerol, a Byproduct of Biodiesel Production,
for Swine

by
Michael Christopher Shields

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APPROVED BY:

Dr. Eric van Heugten
Committee Chair

Dr. Charles Stark

Dr. Jack Odle

Biography

Michael Christopher Shields was born on October 17, 1984 in Norfolk, VA and was raised in Ahoskie, NC. He graduated from Hertford County High School in 2003. He received his Bachelor of Science degree in Animal Science from North Carolina State University in 2007. In the fall of 2007 he re-enrolled in North Carolina State University to obtain a Master of Science degree in Animal Science/Nutrition.

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Chapter 1: Literature Review

Introduction

There has been a dramatic increase in the amount of biofuels produced in the United States which has caused an increase in byproducts, such as glycerol and dried distillers grains. There has been limited research to explore the effects of glycerol on the performance in nursery pigs and the effect of glycerol on the feed manufacturing process. This literature review will focus on the components of biodiesel production, the biochemistry of glycerol, industrial uses of glycerol, impact of glycerol on feed milling, and the potential use of glycerol in livestock species.

Biodiesel Production

Biodiesel has been produced for over 100 years, beginning when Rudolf Diesel tested the use of vegetable oil as a fuel in his engine (Ma and Hanna, 1999). With the evolution of the biodiesel process, as well as the recent increase in both fuel prices and the need for alternative fuels, biodiesel has been used more frequently (Ma and Hanna, 1999). Extensive lab and road tests have shown that the performance of biodiesel is similar to petroleum products, while reducing the emissions of hydrocarbons and carbon monoxides that have been shown to be harmful to the environment. Biodiesel use has increased around the United States in towns, school districts, businesses, and government agencies. Biodiesel production and the number of biodiesel plants have also increased rapidly around the world (Figures 1 and 2). In 2003, there were approximately 450 million gallons of biodiesel produced in Europe (Haas et al., 2006), and the United States produced 500 million gallons in 2008 (National Biodiesel Board). The cost of biodiesel products depends on the product from

which it is derived. It costs around \$1.14/gallon if the fuel is produced from soybeans, but it is more expensive, \$2.62/gallon, if biodiesel is produced with rapeseed (Haas et al., 2006). Biodiesel usually contains twenty percent diesel fuel in a blend, and according to the National Biodiesel Board, it costs twenty cents more on average than diesel fuel (National Biodiesel Board).

The primary production method of biodiesel is transesterification. It is a process where a fat or oil is mixed with an alcohol to form esters and glycerol, which can be referred to as crude glycerin (Ma and Hanna, 1999). Every gallon of biodiesel produced yields approximately 0.66 pounds of glycerol (Thompson and He, 2006). The reaction is reversible and when the esters are being formed, a catalyst and extra alcohol are used to force the reaction forward. The alcohols used in the reaction are primary and secondary monohydric aliphatic alcohols that have between one and eight carbons. Examples of these alcohols include methanol, ethanol, propanol, butanol, and amyl alcohol. Methanol is the major alcohol that is used because it is readily available, polar, and has the shortest chain length of the alcohols. Methanol also reacts quickly with NaOH, which is one of the possible catalysts, and NaOH easily dissolved in methanol (Ma and Hanna, 1999). One of the most important characteristics of this reaction is the effect of the molar ratio. The stoichiometric ratio for transesterification is three moles of alcohol and one mole of triglyceride. Because transesterification is an equilibrium reaction, the higher the ratio of alcohol to triglycerides, the more readily the reaction will proceed. A molar ratio of six alcohols to one triglyceride will provide the most esters which increases the yield of biodiesel. A problem with having a higher ratio (greater than 9:1) is that the oil will interfere with the separation of glycerol from

the esters. This happens because there is an increase in solubility with glycerol and the alcohol. When glycerol is still in the solution, it forces the reaction to the opposite direction which lowers the amount of esters (Meher et al., 2004). NaOH and KOH are the catalysts used in the reaction. When the reaction is catalyzed by an alkali, the glycerides and alcohol must be anhydrous because water will produce soaps which will significantly lower the amount of esters produced. The soaps also make it more difficult to separate the glycerol and the esters. Glycerol is normally recovered because of its value in pharmaceutical drugs, foods, and other major products in the market. Glycerol is recovered by gravity or centrifuging (Ma and Hanna, 1999).

Transesterification occurs in several steps in a cascade of reversible reactions. The triglyceride is broken down to a diglyceride which is then broken down to a monoglyceride and then finally to a glycerol. Through each step, a mole of ester is produced. When an alkali catalyst is added, the reaction is formed in one less step. In the first step, the alcohol anion attacks the carbonyl carbon of the triglyceride which forms a tetrahedral intermediate. During the second step the tetrahedral intermediate reacts with the alcohol, usually methanol, to reproduce the alcohol that was used in the first step. Finally, the tetrahedral intermediate is rearranged which forms a fatty acid ester and a diglyceride (Ma and Hanna, 1999).

Glycerol Metabolism

Glycerol is a three carbon compound that is usually seen in the backbone of triglycerides. It is naturally produced in the body and appears in and around all cells in low concentrations ($<0.1\text{mmol/L}$) (Robergs and Griffen, 1998). Glycerol is formed from a dephosphorylation of glycerol-3-phosphate using glycerol kinase. Glycerol-3-phosphate is

produced from a glycolysis intermediate, dihydroxyacetone phosphate. These reactions are reversible and free glycerol can enter glycolysis and gluconeogenesis (Robergs and Griffen, 1998). When ingested, glycerol is absorbed via Na^+ dependent and secondary active carrier mediated transport in the stomach and the small intestine (Kato et al., 2004). It is absorbed at about one-fourth the rate of glucose (Lin, 1977). Ingestion of an 85% glycerol solution in fasted humans provided an increase in serum glycerol (Sommers et al., 1993). The maximum serum glycerol levels were found to be between one and two hours after glycerol was ingested. The half life of glycerol is reported to be between 0.61 and 1.18 hours (Sommers et al., 1993).

The liver is the main organ responsible for metabolizing glycerol. This is because glycerol is mainly gluconeogenic (Lin, 1977). Three fourths of the body's stores are metabolized in the liver. The kidney is another major organ in glycerol metabolism. It is responsible for utilizing about one fifth of the body's glycerol stores. The kidney can completely clear a serum concentration of about 1 mM (Lin, 1977). Other tissues that have been shown to utilize glycerol are mammary cells, adipose tissue, pneumocytes, the aorta, the heart, and skeletal muscle (Lee et al., 2001).

There are several enzymes relevant in glycerol metabolism. The most studied enzyme is glycerol kinase. Glycerol kinase is found mostly in the liver and the kidneys (Robergs and Griffen, 1998). It is involved in glycerol being phosphorylated to glycerol-3-phosphate (Brisson et al., 2001). The enzyme is stabilized by the amino acid, ethylenediaminetetraacetic acid (EDTA), and by low concentrations of glycerol (Lin, 1977). The enzyme is stable at around a pH of 5, and its activity requires a sulfhydryl group to be

present. ATP is the most common form of energy used, but UTP and CTP can also be used as energy sources (Lin, 1977). If ADP is present, it inhibits the activity of glycerol kinase, while ATP and glycerol increase the activity of glycerol kinase (Grunnet and Lundquist, 1967).

Another major enzyme is glycerol-3-phosphate oxidoreductase. The vast majority of the glycerol-3-phosphate oxidoreductase is found in skeletal muscle with small amounts in the liver and kidneys (Lin, 1977). Glycerol-3-phosphate oxidoreductase has several functions. It produces glycerol-3-phosphate for triglyceride and phospholipid synthesis, helps to maintain cytosolic NAD⁺ level during anaerobic glycolysis, and catalyzes the reaction of glycerol-3-phosphate to dihydroxyacetone phosphate (Zolnierowicz et al., 1985). Glucocorticoids are also important in the regulation of glycerol-3-phosphate oxidoreductase, as the enzyme is reduced dramatically if the adrenal or pituitary gland is removed. Also, if adrenocorticotrophic hormone or cortisol is injected in the animal, the enzyme's activity increases (Lin, 1977).

The third major enzyme in glycerol metabolism is glycerol-3-phosphate dehydrogenase. This enzyme is found throughout the body in the inner mitochondrial membrane. In rats, its highest activity is in the mitochondria of the testis, but it can also be high in the mitochondria of the skeletal muscles, lungs, spleen, intestines and brain (Lin, 1977). In humans, it has been measured in skeletal muscles, the liver, the kidneys, smooth, and cardiac muscle (Brisson et al., 2001). Glycerol-3-phosphate is involved in converting dihydroxyacetone phosphate to glycerol-3-phosphate during gluconeogenesis (Berrada et al., 2001). Thyroid hormones increase the activity of this enzyme in most tissues of the body.

Rat liver mitochondria show an increase in glycerol-3-phosphate dehydrogenase when the adrenal gland is removed (Lin, 1977). A diet high in glycerol also increases this enzyme in the liver and in the kidneys. In cold weather, this enzyme increases in brown adipose tissue of rats while the rat is acclimating itself to the cold (Lin, 1977).

Methanol Metabolism

One of the major concerns with feeding glycerol from biodiesel production to production animals is the amount of methanol in the crude glycerol. Crude glycerol contains residual methanol, and consequently it is important to understand methanol metabolism and how it relates to animal health (Lammers et al., 2008). Methanol can be toxic to animals, so the Food and Drug Administration states that the level should be lower than 150 ppm in feed. It also has a low evaporation point (65°C), so during pelleting it will be lost in the feed mill and could become hazardous to the mill employees (Pluske et al., 2007). After ingestion, absorption of methanol takes place within six hours, even if methanol is ingested or inhaled at high doses. After absorption, the highest concentrations of methanol are found in the liver, kidneys, and the gastrointestinal tract. It is found in the lowest concentrations in the muscle, fat, and the brain. Methanol is excreted through the lungs over several days after ingestion or inhalation (Roe, 1955). In the liver, methanol undergoes oxidation to carbon dioxide and water through the intermediates, formaldehyde and formate. The first intermediate in methanol metabolism is formaldehyde. Formaldehyde is then oxidized to formic acid by glutathione dependent alcohol dehydrogenase which is present in many different tissues of animals and humans. Formic acid then disassociates into formate and hydrogen ions. Formate is removed from the organism slowly through oxidation to carbon dioxide by the

liver. Formate is oxidized quickly in the rat and does not accumulate which is not true with other species (Skrzydłowska, 2003). Formate is converted to 10-formyltetrahydrofolate by 10-HCO H₄ folate synthetase. 10-HCO H₄ folate is then oxidized to carbon dioxide. The main reason that methanol is toxic is because of the formate accumulation in the body. Formate causes toxicity because it disrupts the mitochondrial electron transport chain and energy production. Formate inhibits cytochrome oxidase activity, a key component in the electron transport chain that is involved in ATP synthesis (Treichel et al., 2003). Formate accumulates in species such as monkeys and swine because of their low concentrations of H₄ folate (Maker et al., 1990).

There have been several studies in humans that have reported methanol toxicity. In the early 1900s methanol poisoning was more common when methanol was used in paint and other household items (Medinsky and Dorman, 1995). Methanol poisoning can result in blindness, metabolic acidosis, and death. Medinsky and Dorman, (1995) exposed humans to 200 ppm of methanol for up to six hours while they were resting or doing mild exercise. No changes in serum formate concentrations were observed. The researchers concluded, in agreement with other studies, that exposure to methanol concentrations from 10-200 ppm in vapor via inhalation are safe and do not pose a risk to healthy adults (Medinsky and Dorman, 1995).

There also have been studies conducted in swine. Maker et al. (1990) used six pigs between the weights of 9.5 and 14 kg to compare formate metabolism in swine and rats. The pigs were injected intraperitoneally with sodium formate. After the injection, plasma concentrations were measured over a period of five hours. The disappearance of formate in

the plasma of pigs was much slower than that of rats. Compared to rats, the concentration of H₄ folate was much lower in pigs than rats which indicate that swine may be more sensitive to methanol. Another study by Dorman et al. (1993) used fourteen four month old female minipigs and gave an oral dose of 0, 1, 2.5, or 5 g/kg of methanol. The researchers also gave a formate buffer (425 g/kg) to two additional minipigs to determine if formate accumulation alone would cause neuro-ocular toxosis. Acute toxicosis developed between thirty minutes to two hours after methanol was given, and the symptoms of toxicosis were gone by fifty two hours after the administration of the methanol. The signs of methanol toxicosis were central nervous system depression, ataxia, recumbency, and tremors. Severity of symptoms increased with increasing levels of methanol. After the symptoms disappeared, the minipigs did not have a reoccurrence of the symptoms at any time until they were euthanized. In addition to the above symptoms, the minipigs that were administered the intravenous formate also developed depression, polyuria, and polydipsia between thirty six and forty hours after the dose, but neither animal developed metabolic acidosis or formate accumulation (Dorman et al., 1993).

Industrial Uses of Glycerol

Glycerol is valuable in many different industries as it is used in over 1,500 products. The main uses are in candy, cake mixes, medicines, lotions, shampoo, soaps, detergents, and makeup. It is used in the industrial world in emollients, lubricants, solvents and chemical dispersing products. In the United States, it is used as a lubricant in cough syrups, toothpaste and many other personal hygiene products. It is also used as an artificial sweetener to replace sugar in low-fat foods (SRI consulting, 2004). When biodiesel production increased,

there was a large increase in glycerol which in turn dropped the price of glycerol dramatically. In October of 2008, crude glycerin was trading at five to six cents a pound. The high in 2008 was twenty-five cents a pound which occurred during the summer when the commodity prices increased as well. The low was in September of 2008 when glycerol traded at one cent per pound. FC-Stone, which is a commodity risk management company, projected that six hundred million gallons of biodiesel would be produced in 2008. This would result in an extra sixty million gallons of glycerol on the market which could keep the price low for some time.

Impact of Glycerol on Feed Milling

Glycerol can have advantages and disadvantages in the feed mill. If too much is added, the glycerol can cause the feed to be sticky and lower the flow of feed through the mill, cause problems in feeders, or cause buildup in the pellet mill. Cerrete et al. (2006) included 10% glycerol in broiler diets and reported lower pellet quality with the 10% glycerol diets and a negative effect on the flow of feed in the feeders. Groesbeck et al. (2008) performed more extensive feed mill studies and added up to 15% glycerol into the feed. The researchers collected pellet mill production data which included pellet mill electrical consumption, production rate, hot-pellet temperature, motor load, feeder rate, conditioning rate, and pellet durability. Conditioning was held constant at 65.5°C, and there was no effect of crude glycerol on conditioning temperature. Hot pellet temperature decreased linearly as crude glycerol in the diet increased. Delta temperature, the difference between hot pellet temperature and conditioning temperature, decreased linearly as crude glycerol increased. This linear decrease indicates a reduction of friction of feed passing

through the pellet die with increasing levels of glycerol. There was no difference in voltage between treatments. Amperage and motor load decreased when glycerol was added with the greatest reductions at 3% and 12% added glycerol. By decreasing amperage and motor load, the feed mill would be able to save a significant amount of money on energy costs. Pellet durability index (PDI) increased with increasing crude glycerol (Groesbeck et al 2008). In another study, Groesbeck et al. (2007) measured flowability of diets with 0, 2, 4, 6, or 8% of added glycerol and found that glycerol improved flowability (Groesbeck et al., 2007).

Glycerol Use in Ruminants

When glycerol is fed in the diet of ruminants, it is fermented to volatile fatty acids (VFA) in the rumen. Feeding of glycerol at increasing levels has been shown to increase the levels of propionate and butyrate and to decrease the levels of acetate (Redmond et al., 1993). It takes several days for the rumen microbial population to adapt to the feeding of glycerol, but after the initial period, glycerol disappears within six hours (Redmond et al., 2003). There is contradicting information for in vivo disappearance. Kijora et al. (1998) as cited in Redmond et al. (2003) fed 200 g of and 85% of the glycerol disappeared within two hours in cattle that were adjusted to glycerol (Kijora et al., 1998). Redmond et al. (2003) fed 240 g of glycerol to cattle that had been adjusted to glycerol and reported disappearance rates of 1.2 to 2.4 g/hour.

Glycerol was first fed to cattle as a treatment for ketosis in the 1950s and it was studied further in the 1970s (Donkin and Doane, 2007). In the last decade, it has been studied more as a treatment for metabolic problems that occur in transition dairy cows (Donkin and Doane, 2007). Defrain et al. (2004) fed two levels of glycerol (1.43 kg and 0.86

kg) and a control diet with no glycerol per day to transition dairy cattle. Rumen fluid collected from postpartum cows showed a greater total of volatile fatty acids (70.2 and 61.4 for cows fed glycerol vs. 56.2 for cows fed no glycerol) in the cows that received glycerol. Cows fed glycerol had a lower ratio of acetate to propionate and had higher plasma glucose concentrations. No changes were observed in body weight, condition loss, plasma nonesterified fatty acids, and liver lipids (Defraín et al., 2004). Milk production decreased in cows fed glycerol, and glycerol also decreased urea nitrogen concentrations in milk compared to the control diet (Defraín et al., 2004).

Fisher et al. (1973) fed low levels of glycerol to beef cattle and found no difference in DMI. However, Donkin and Doane (2007) fed up to 15% glycerol in the diets of beef cattle and showed an increase in DMI. Similarly, Khalili (1997) observed increased DMI when feeding 0.56 lb/d of glycerol to beef cattle. Elam et al. (2008) evaluated three levels of glycerol (0, 7.5, and 15%, replacing steam-flaked corn) in feedlot beef cattle and reported reduced performance with glycerol due to a linear decrease in DMI. In a second study, cattle consumed more feed with 10% glycerol than the control diet without glycerol; however this had no effect on carcass characteristics, performance, or intake (Elam et al., 2008). Parsons et al. (2008) reported a linear increase in DMI in heifers fed 0, 2, 4, 8, 12, and 16 % crude glycerol as glycerol levels increased. When fed at levels of 8% and lower, glycerol increased body weight. Longissimus muscle area, subcutaneous fat over the 12th rib, and marbling scores all decreased linearly as glycerol level increased (Parsons et al., 2008).

Glycerol Use in Poultry

Glycerol use in poultry has been studied since the 1960s. Campbell and Hill (1966) found no improvement in performance of poultry fed high concentrations of glycerol in a low fat diet. Lammers et al. (2008) studied the metabolizable energy (ME) value of crude glycerol for laying hens using four treatments: 0, 5, 10, and 20% glycerol. No differences were observed in egg production rates, egg weight, egg mass or feed consumption. Apparent ME value of crude glycerol in that study was calculated to be $3,805 \pm 238$ kcal/kg for laying hens. Dozier et al. (2008) assessed the apparent metabolizable energy of crude glycerol for broiler chickens. They estimated apparent ME to be 3,621, 3,331, and 3,349 kcal/kg for chicks that were 4-11, 17-25, and 37-45 days of age respectively (Dozier et al., 2008). Cerrate et al. (2006) performed two experiments using glycerol as a feed ingredient in broiler chickens examining performance and carcass characteristics. In the first experiment glycerol was fed at 0, 5, and 10% of the diet. No differences were seen in birds fed 0 or 5% glycerol, but birds fed 10% glycerol had decreased body weight, ADFI, dressing percentage, and weight of breast meat, wings and leg quarters. In experiment 2, birds were fed 0, 2.5, and 5% glycerol in the diet and birds fed 2.5 and 5% glycerol had higher body weight, breast yield and lower wing yield compared to the control diet (Cerrate et al., 2006).

Glycerol Use in Swine

Lammers et al. (2008) performed a study to determine the apparent DE and ME of crude glycerol in pigs and reported that the crude glycerol in this study had a DE of $3,344 \pm 8$ kcal/kg and a ME of $3,207 \pm 10$ kcal/kg, which indicates that glycerol is a suitable energy source for starter or finishing pigs (Lammers et al., 2008).

There have been several studies that have assessed performance of pigs fed glycerol. Groesbeck et al. (2008) used six different treatment diets that contained 0, 3, 6, 9, 12, and 15% crude glycerol. Pigs fed increasing levels of glycerol had a higher ADG and ADFI than pigs that had no glycerol in the diet, but increasing levels of glycerol had no effect on gain: feed (G:F) (Groesbeck et al., 2008). Lammers et al. (2008) fed pigs 0, 5, 10% crude glycerol and found no difference in ADG, ADFI, and G: F. Casa et al. (2009) reported no differences in pigs fed 5% pure glycerol but observed reduced ADG and G:F in pigs fed 10% pure glycerol. Kijora et al. (1995), as cited in Pluske (2007), fed 5, 10, 20, and 30% glycerol to finishing pigs and found that the pigs fed 5 and 10% had a better ADG and ADFI. The pigs fed 30% glycerol had similar ADG or ADFI but had improved G:F. Kijora et al. (1995) recommended that pigs can be fed up to 10% glycerol in the diet (Pluske 2007). A limit of 10% was recommended because feeding 30% glycerol in the feed can cause flowability problems. Mourot et al. (1994) fed two levels of glycerol (0 or 5%) and found no significant differences in growth performance, but the study did find a slightly higher G:F and lower ADG in pigs fed glycerol (Mourot et al., 1994).

Several studies have examined the carcass characteristics and the fatty acid profile after glycerol has been added to the diet. Lammers et al. (2008) reported that adding up to 10% glycerol to the diet did not affect carcass drip loss, 10th-rib backfat, longissimus muscle area, percent fat free lean, meat quality, or sensory evaluation. Pigs fed 10% glycerol had lower concentrations of linoleic acid in the longissimus muscle, while eicosapentaenoic acid in the longissimus muscle increased as glycerol in the diet increased. Casa et al. (2009) fed pure glycerol and observed no differences in dressing yield, pH, drip losses, lean meat

content, and weight and yield of lean cuts. Mourot et al. (1994) reported an increase in carcass drip loss in longissimus dorsi and semimembranosis muscles of animals that were fed glycerol. This contradicts the work done by Lammers et al. (2008) and Casa et al (2009) that found no effects of glycerol on drip loss. Mourot et al (1994) reported that the plasma cholesterol level increased in pigs fed glycerol. This confirms previous results from Lin (1977) who had similar findings in other species. Glycerol did not affect lipid contents of backfat, semimembranosis muscle, and liver tissue, but it decreased the proportion of linoleic and linolenic acid in backfat (Mourot et al., 1994). Oleic acid in backfat and intramuscular fat also decreased with glycerol (Mourot et al., 1994).

Kijora and Kupsch (1996) evaluated the effects of 10% pure glycerol, 5% and 10% crude glycerol from one source, and 5 and 10% crude glycerol from a second source compared to a control diet in growing-finishing pigs. Pigs fed glycerol had a higher ADFI which led to a higher ADG in the growing phase, regardless of the source, but not the finishing phase. Glycerol did not impact carcass characteristics, and there was no difference in the performance of pigs fed pure glycerol and crude glycerol (Kijora and Kupsch 1996).

Summary

With the high amount of biodiesel being produced causing an abundance of glycerol on the market, it is important to find alternate uses of glycerol. This review has shown that there are many uses for glycerol in the livestock industry, but research is still limited. The purpose of the current research project was to examine the use of glycerol in nursery pig diets and the impact of glycerol in feed milling.

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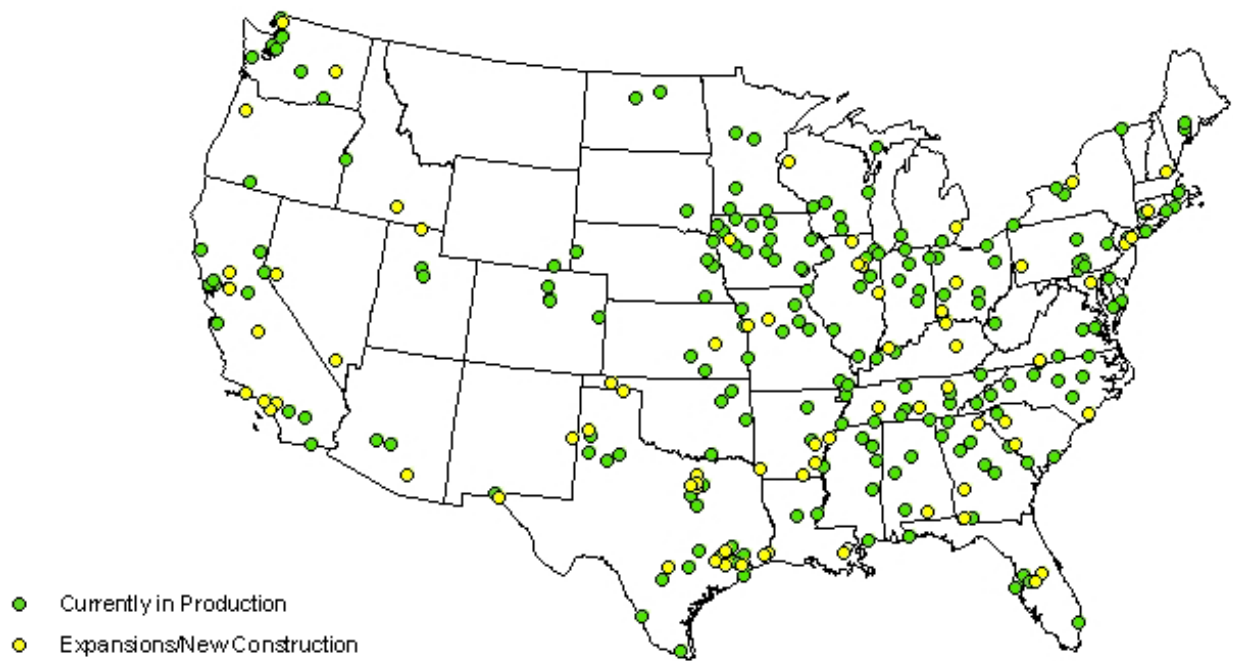
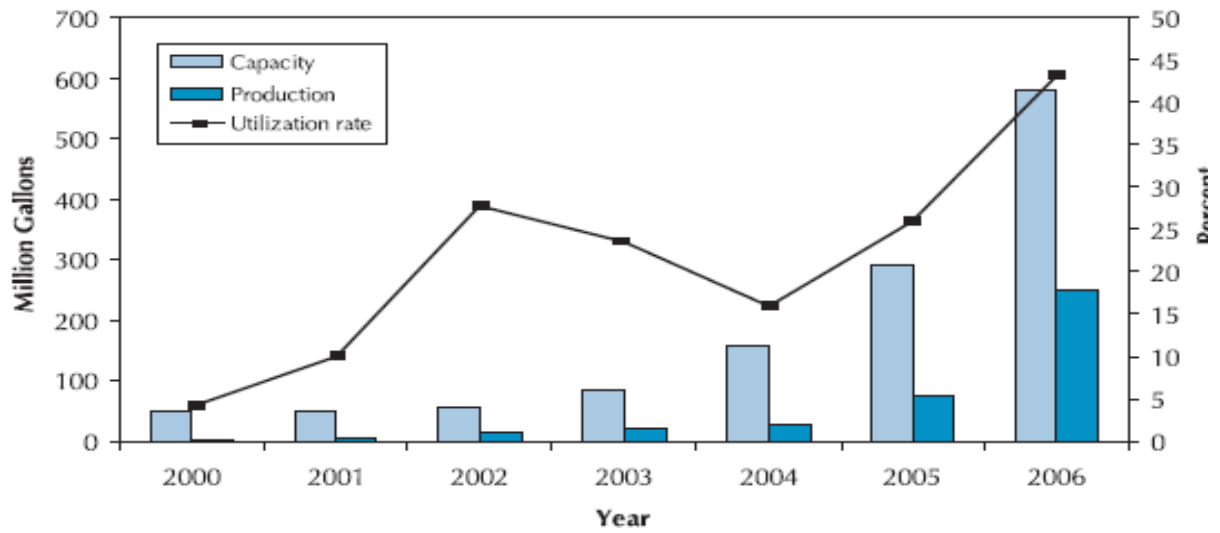


Figure 1: Biodiesel Plants in the United States in 2008 (Centers for Agricultural and Rural Development)



Source: National Biodiesel Board.

Note: Capacity given is on September 1 of each year.

Figure 2: Biodiesel production from 2000-2006 in the United States (National Biodiesel Board)

CHAPTER 2: Evaluation of the Nutritional Value of Glycerol, a Byproduct of Biodiesel Production, for Swine

Abstract

Four experiments were completed to evaluate the effects of supplementing glycerol on pellet mill efficiency and nursery pig performance. Two experiments were performed to test the performance of glycerol in the feed mill. In Experiment 1, finisher feed was mixed in a 50 kg mixer at glycerol levels of 0, 2.5, and 5%. Diets were manufactured in batches of 100 lbs in a randomized block design, and this was replicated three times. In Experiment 2, finisher feed was mixed and pelleted to examine feed mill performance at glycerol levels of 0, 2.5, and 5%. Diets were manufactured in batches of 1,000 lbs in a randomized block design, and this was replicated three times. In Experiment 1, as glycerol levels increased (0, 2.5 and 5%) flowability increased ($P = 0.03$) linearly (26.53, 25.2, 23.73 mm disc size respectively). In Experiment 2, pellet mill efficiency linearly increased ($P = 0.01$) by 27% and pellet durability linearly increased ($P = 0.004$) by 46% as glycerol levels increased. Hot pellet temperature decreased ($P = 0.05$) by 2% and delta temperature also decreased ($P = 0.02$) by 30% as glycerol levels increased. In Experiment 3, a total of 126 pigs (body weight was 6.68 ± 0.17 kg) was weaned at approximately 21 days of age, blocked by weight, and allocated to 42 pens with 3 pigs per pen. Pens were randomly assigned one of six treatments in a 2x3 factorial randomized complete block design with factors: 1) glycerol inclusion in phase 1 diets (0 or 5%) and 2) glycerol inclusion level in phase 2 diets (0, 5, or 10%). Phase 1 diets were fed for 2 weeks, and glycerol was supplemented to replace lactose on a weight-for-weight basis. Phase 2 diets were fed for 3 weeks, and glycerol was included in

replacement of corn. This replacement was made on a nutrient basis (thus accounting for the nutrient composition of corn). Results showed that replacing corn at levels of up to 10% improved ADG ($P<0.002$) by 16%, ADFI ($P<0.003$) by 13%, and G:F ($P<0.044$) by 2%. Serum glycerol concentration was not impacted by the glycerol supplementation during the starter 1 phase, but a 16% linear increase in serum glycerol concentration during the starter 2 phase ($P<0.0001$) was observed as dietary glycerol increased. Glycerol supplementation had no effect on serum glucose, total protein, albumin, bilirubin, creatine phosphokinase, globulin, and aspartate aminotransferase in the starter 1 phase. In the starter 2 phase, glycerol supplementation increased urea nitrogen ($P=0.002$), decreased creatinine ($P=0.02$), and increased the ratio of blood urea nitrogen to creatinine ($P=0.0002$). Glycerol supplementation in the starter 2 phase had a quadratic effect on urea nitrogen ($P=0.0008$), which was lower at 5% level of glycerol (4.71mg/dl) compared to the 0 and 10% (6.86 and 7.43mg/dl, respectively). Cholesterol concentration was higher for 5% added glycerol (92.21mg/dl) than for the 0% and 10% levels (79.14 and 84.64mg/dl respectively; quadratic effect, $P = 0.04$). Glycerol supplementation in the starter 2 phase linearly decreased creatinine concentration ($P=0.02$).

In Experiment 4, a total of 144 pigs (body weight was 6.68 ± 0.17 kg) were weaned at approximately 21 days of age, blocked by weight, and allocated to 28 pens with 3 pigs per pen. Pens were randomly assigned one of six dietary treatments (0, 2.5, 5, 7.5, 10% glycerol added to replace 10% lactose in a basal starter 1 diet (fed for two weeks) containing 20% total lactose and a negative control with 10% lactose and 0% glycerol). A common starter diet was fed for the remaining two weeks. Pigs were weighed and feed intake was measured

weekly. Results demonstrated that glycerol supplementation at 10% compared to the negative control resulted in a greater ADG during weeks 1, 2, and the starter 1 period ($P = 0.03$) with ADG highest in pigs fed 10% glycerol increased by 35%. Glycerol also improved feed efficiency in the starter 1 period ($P = 0.04$). There was no impact of feeding glycerol in the starter 1 phase on subsequent performance during the starter 2 phase. Serum glycerol was linearly increased (7.1, 7.5, 31.1, 128.2, and 97.0 mg/dl) ($P = 0.03$) as glycerol levels increased. Glycerol supplementation in starter 1 diets had no effect on glucose, urea nitrogen, total protein, albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, calcium, phosphorus, sodium, potassium, chloride, albumin, globulin, the ratio of blood urea nitrogen to creatinine (buncreatinine), or creatine phosphokinase. Glycerol supplementation decreased creatinine ($P = 0.0004$) and bilirubin ($P = 0.02$) as dietary glycerol levels increased. Overall these studies indicate that glycerol can be an asset in feedmilling by improving pellet durability, flowability, pellet mill efficiency, and hot pellet temperature. In nursery diets, it improves ADG and ADFI at levels up to 10%.

Introduction

In the United States, the production of biodiesel has increased dramatically, from 30 million gallons in 2004 to 500 million gallons in 2008 (National Biodiesel Board, 2009). Biodiesel is produced by transesterification of a fat or oil that is mixed with an alcohol, usually methanol, using a catalyst (NaOH or KOH) to form esters and glycerol (Ma and Hanna, 1999). For every liter of biodiesel produced, 79 grams of glycerol is generated (Thompson and He, 2006).

Glycerol is used in over 1,500 products such as candy, cake mixes, medicines, lotions, soaps, detergents, and cosmetics. In the industrial world it is used in emollients, lubricants, solvents and chemical dispersing products. Crude glycerol contains approximately 80 to 88% glycerol with the remaining 12-20% being water and salt. Pure glycerol contains 99.5% glycerol (National Biodiesel Board).

Glycerol has been studied as a feed ingredient for pigs for several years, and it has been shown to be highly palatable, increasing ADG and ADFI at levels up to 10% in nursery pigs (Groesbeck et al., 2008) and grower/finisher pigs (Kijora et al., 1995; Kijora and Kupsch, 1996). Other studies have shown no differences in ADG, ADFI, or G:F in grower/finisher pigs with levels up to 10% (Lammers et al., 2008; Mourot et al., 1994). Casa et al. (2009) found no differences in ADG, ADFI, and G:F at 5%, but decreased ADG, ADFI, and G:F were found at levels of 10%. Lammers et al. (2008) reported an apparent DE of 3,344 kcal/kg and an ME of 3,207 kcal/kg in pigs.

Cerette et al. (2006) reported that the addition of 10% glycerol in broiler diets lowered pellet quality and had a negative effect on the flowability of feed. Groesbeck et al. (2008) found no effect of glycerol on conditioning temperature. The researchers found linear decreases in hot pellet temperature, delta temperature, amperage and motor load when glycerol was added at increasing levels to 15%. Pellet durability (Groesbeck et al., 2008) and flowability (Groesbeck et al., 2007) has been found to increase with increasing levels of glycerol.

The objectives of the present study were to determine: 1) diet manufacturing characteristics and feed mill processing data when using glycerol, 2) the value of glycerol in

diets for newly weaned pigs relative to lactose, and 3) the value of glycerol in diets for nursery pigs when replacing lactose and corn.

Materials and Methods

Feed Manufacturing

Preliminary Experiments

Two preliminary experiments were conducted to determine the maximum inclusion level of glycerol when applied to corn (preliminary Experiment 1) or a complete prestarter diet containing plasma protein, fish meal, and whey (preliminary Exp 2). The addition of 10% glycerol to corn did not appear to cause problems in terms of flowability. In Exp 2, spraying glycerol onto a complete prestarter feed (pelleted) appeared to make the pellets stickier, and a level of 6 to 8% resulted in a product that maintained good flow characteristics. The addition of 20% glycerol caused pellets to congeal and form a solid mass. Based on these preliminary studies, the maximum inclusion of glycerol in subsequent studies did not exceed 10%.

Experiment 1

Three finisher diets were formulated to contain three levels of glycerol (0, 2.5, and 5%) (Table 1). The crude glycerol was the same as used in Lammers et al. (2008), and it contained 86.95% total glycerol, 9.22% moisture, 1.26% sodium, 1.86% chloride, and 280 ppm of methanol. Diets were manufactured in batches of 454.54 kg in a randomized block design, and this was replicated three times.

Feed was manufactured at the North Carolina State University Feed Mill Educational Unit in accordance with current Good Manufacturing Processes. Corn was ground with a

hammer mill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with a 2.2 mm (6/64 in) screen. Feed was mixed in a Davis precision horizontal paddle mixer (HD1 mixer H.C. Davis Sons Manufacturing Co., Inc, Bonner Springs KS). Samples were obtained at the mixer discharger. Flowability was determined using a Flowdex flow meter (Hanson Research Corporation, Chatsworth, CA). A 50g sample was used to fill the cylinder to within 1cm from the top, 30 seconds passed before starting the test. A 16mm flow disk was used to start the test. Positive test results were seen if the hole was visible. The test was repeated until no hole was visible and the lowest positive result was recorded. After a positive test was confirmed, the test was repeated to verify the result.

Experiment 2

Three finisher diets were formulated to contain three levels of glycerol (0, 2.5, and 5%) (Table 1). The crude glycerol that was used was the same as in Experiment 1. Diets were manufactured in batches of 454.45kg in a randomized block design, and this was replicated three times. Two conditioning temperatures were used for pelleting the diets. All diets were pelleted at a conditioning temperature of 74°C, because glycerol containing diets, especially at 5% inclusion, could not be pelleted at higher temperatures. In addition, a second batch of control feed was pelleted at 85°F, which is a more common pelleting temperature used for swine diets. In all, there were four treatment comparisons.

Feed was manufactured at the North Carolina State University Feed Mill Educational Unit in accordance with current Good Manufacturing Processes. Corn was ground with a hammer mill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with a 2.2 mm (#6) screen. Dry ingredients were blended in a double ribbon mixer, and liquids (fat and glycerol)

were added after dry mixing was complete. Pellets were manufactured using a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) equipped with a 4.4 mm x 25 mm die (11/64 in x 1 in). Pellets were cooled with ambient air in a counter-flow cooler (Model VK09x09KL, Geelen Counterflow USA Inc., Orlando, Florida). Electrical consumption was recorded on the pellet mill main motor. Conditioning temperature was recorded during the pelleting process. Samples were collected immediately after the pellet die to determine the hot pellet temperature and pellet durability index (PDI). Conditioning temperature was measured by a thermometer placed in the stream of the conditioned mash between the end of the conditioner and the pellet die. Hot pellets were collected in an insulated pail and measured with a thermometer after the temperature reached equilibrium. Delta temperature is measured as the difference between hot pellet temperature and conditioning temperature. Pellet mill efficiency was calculated based on production rate and horsepower readings. Standard durability index (PDI) was evaluated for each replicate using 500 g of cold pellets (ASAE Standard S 269.4; 2007). Moisture content of the mash feed sample prior to pelleting, after condition, and of the cooled pellets was determined (ASAE Standard S 352.2; 2007).

Pig Performance

Experiment 3

A total of 126 pigs (body weight was 6.91 ± 0.18 kg) were weaned at approximately 21 days of age at the Swine Educational Unit, Raleigh, NC. Pigs were weighed and assigned within sex and weight block to one of 6 dietary treatments. Pigs were housed 3 pigs per pen using 42 pens, and there were 7 replicates per treatment. Dietary treatments (Tables 2 and 3)

were arranged in a 2 x 3 factorial randomized complete block design. Factors consisted of: 1) glycerol inclusion in phase 1 diets (0 or 5%) and 2) glycerol inclusion level in phase 2 diets (0, 5, or 10%). Glycerol supplementation in Phase 1 diets replaced lactose on a weight for weight basis. Glycerol inclusion in phase 2 diets was made in replacement of corn and this replacement was made on a nutrient basis (thus accounting for the nutrient composition of corn). Crude glycerol was the same as used in experiments 1 and 2.

Feed was manufactured at the North Carolina State University Feed Mill Educational Unit in accordance with current Good Manufacturing Processes. Corn was ground with a hammer mill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with a 2.2 mm (6/64 in) screen. Dry ingredients were blended in a double ribbon mixer and liquids were applied after dry mixing was complete. Pellets were manufactured using a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) equipped with a 4.4 mm x 45 mm die (11/64 in x 1 3/4 in). Pellets were cooled with ambient air in a counter-flow cooler (Model VK09x09KL, Geelen Counterflow USA Inc., Orlando, Florida). Post-pelleting liquid application of glycerol was completed after the cooling process. Samples were collected immediately after the pellet die to determine hot pellet temperature and pellet durability index.

A basal diet was created for both phase 1 and phase 2 diets. For phase 1 diets, all ingredients, except for glycerol and the lactose it replaced were added to the mixer to create a basal diet. The basal diet was then split into two portions to which the appropriate levels of glycerol or lactose were added. Additional salt and water were added to the lactose containing diet to maintain the same level of salt and water as the glycerol containing diets.

Pigs were fed a two-phase dietary program. The first phase diet was fed immediately following weaning for 14 days. Glycerol was included in these diets as indicated above in replacement of lactose. The second phase diet did not contain any lactose, and therefore, glycerol was included in replacement of corn on a least cost basis. The second phase diet was fed for 3 weeks.

Pigs were weighed weekly on an individual basis throughout the 5 week experimental period. Feed added to the feeders was recorded, and feeders, including any remaining feed, were weighed weekly to determine feed disappearance. Serum samples were taken from pigs on days 13 and 27 by venipuncture into 10 ml vacuum tubes (BD Vacutainer, Franklin Lakes, NJ). Samples were centrifuged at 900 x g for 20 min at 25°C. Serum was collected and stored at -20°C pending analysis. A commercially available kit (Sigma Chemical Co., St. Louis, MO) was used to measure serum glycerol concentrations (F6428). Serum samples were sent to Antech Diagnostics Lab (Lake Success, NY) and analyzed for glucose, urea nitrogen, creatinine, total protein, albumin, bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, cholesterol, calcium, phosphorus, sodium, potassium, chloride, albumin, the ratio of blood urea nitrogen to creatinine (bun:creatinine), globulin, and creatine phosphokinase (CPK).

Experiment 4

A total of 144 pigs (body weight was 6.68 ± 0.17 kg) were weaned at approximately 21 days of age at the Swine Educational Unit, Raleigh, NC. Pigs were weighed and assigned within sex and weight block to one of 6 dietary treatments. Pigs were housed 3 pigs per pen

using 48 pens and resulting in 8 replicates per treatment. Dietary treatments (Table 3) consisted of the following:

- 1) Control treatment containing 20.0% lactose and 0% glycerol
- 2) Diet with 17.5% lactose and 2.5% glycerol
- 3) Diet with 15.0% lactose and 5.0% glycerol
- 4) Diet with 12.5% lactose and 7.5% glycerol
- 5) Diet with 10.0% lactose and 10.0% glycerol
- 6) Diet with 10.0% lactose and 0.0% glycerol

The control diet contained 20% total lactose; therefore, glycerol replaced up to 10% of the lactose. A second control diet was included in the design that contained 10% lactose and no added glycerol. Replacement of lactose with glycerol was conducted on a weight for weight basis because the DE of lactose and glycerol were expected to be similar. The crude glycerol used in Experiment 4 was the same as what was used in Experiment 3.

Feed was manufactured at the North Carolina State University Feed Mill Educational Unit in accordance with current Good Manufacturing Processes. Corn was ground with a hammer mill (Model 1522, Roskamp Champion, Waterloo, IA) equipped with a 2.2 mm (6/64 in) screen. Dry ingredients were blended in a double ribbon mixer and liquids were applied after dry mixing was complete. Glycerol was added to the diets with the dry ingredients prior to pelleting (which is in contrast to Experiment 3 in which glycerol was applied post-pelleting). Pellets were manufactured using a pellet mill (Model PM1112-2, California Pellet Mill Co., Crawfordsville, IN) equipped with a 4.4 mm x 25 mm die (11/64

in x 1 in). Pellets were cooled with ambient air in a counter-flow cooler (Model VK09x09KL, Geelen Counterflow USA Inc., Orlando, Florida).

Diets were manufactured by creating a basal diet first that contained all ingredients with the exception of lactose and glycerol. The basal was divided into 6 portions to which lactose (with additional salt and water) and glycerol were added to create the final dietary treatments. This ensured that diets were identical in their composition with the exception of lactose and glycerol content.

Pigs were fed a two-phase dietary program. The first phase diet (Table 4) was fed immediately following weaning for 14 days. Glycerol was included in these diets as indicated above in replacement of lactose. Pigs were fed a common second phase diet (Table 5) to evaluate potential carry-over effects of glycerol feeding in the first diet phase. The second phase diet was fed for 2 weeks.

Pigs were weighed weekly on an individual basis throughout the 4 week period. Feed added to the feeders was recorded and feeders with remaining feed were weighed weekly to determine feed disappearance. Serum samples were taken from pigs on day 13 by venipuncture into 10 ml vacuum tubes (BD Vacutainer, Franklin Lakes, NJ). Samples were centrifuged at 900 x g for 20 min at 25°C. Serum was collected and stored at -20°C pending analysis. A commercially available kit (Sigma Chemical Co., St. Louis, MO) was used to measure glycerol (F6428). Serum samples were sent to Antech Diagnostics Lab (Lake Success, NY) and analyzed as described for Experiment 3.

Statistical Analysis

Statistical analysis was performed using the GLM procedure (SAS Inst. Inc., Cary, NC). The model for Experiment 1 included replication and glycerol levels. Orthogonal contrast comparisons were made to determine linear and quadratic effects of glycerol inclusion. The model for Experiment 2 included replication and glycerol levels. Orthogonal contrast comparisons were made to determine linear and quadratic effects of glycerol inclusion. Also, single degree of freedom contrast comparisons were conducted to determine the effect of conditioning temperature (165 vs. 185). The model for Experiment 3 included weight block, glycerol levels in phase 1 diets, glycerol levels in phase 2 diets, and the interaction between level in phase 1 and phase 2 diets. Orthogonal contrast comparisons were made to determine linear and quadratic effects of glycerol inclusion in phase 2 diets. The model for Experiment 4 included weight block and dietary treatment. Orthogonal contrast comparisons were made to determine linear and quadratic effects of glycerol inclusion. In addition, single degree of freedom contrast comparisons were conducted to determine the effect of lactose (10 vs. 20% lactose; treatment 1 vs. 6) and the effect of glycerol (0 vs. 10%; treatment 5 vs. 6) in a basal diet containing 10% lactose.

Results

Feed Manufacturing

Experiment 1

Glycerol supplementation at 0, 2.5, and 5% (26.53, 25.2, and 23.73 mm disc size, respectively) linearly increased flowability of corn-soybean meal based mash diets ($P = 0.03$) (Table 6).

Experiment 2

There was no impact of glycerol supplementation on production rate, moisture, or conditioning temperature. Also, there was no impact of conditioning temperature used for pelleting the two control diets on production rate or horsepower (Table 7). Glycerol supplementation linearly increased pellet durability ($P = 0.004$) resulting in a 46% improved in pellet durability as determined by pellet durability index. Pellet mill efficiency improved by 27% (linear $P = 0.01$) as glycerol supplementation increased. Hot pellet temperature decreased linearly ($P = 0.05$) by 2% ($P = 0.05$) and delta temperature also decreased ($P = 0.02$) by 30% as glycerol levels increased (Table 7). Glycerol supplementation had a tendency to decrease horsepower ($P = 0.06$) (Table 7). Increasing conditioning temperature from 74°C to 85°C provided an improvement in pellet durability index ($P = 0.0002$), improving pellet durability by 42% and a higher hot pellet temperature ($P < 0.0001$) by 8%. Conditioning temperatures that were measured were exactly as targeted (Table 7).

Pig Performance

Experiment 3

Inclusion of 5% glycerol in starter 1 diets had no effect ($P = 0.29$) on body weight, ADG, ADFI or G:F (Table 8). An interactive effect between glycerol inclusion in starter 1 and starter 2 ($P = 0.04$) was noted for pig body weight on week 2 (this interaction was not relevant because starter 2 diets had not been fed at that point) and week 5 of the study. In week 5, there was an interaction due to the low value (18.50 kg) for pigs fed 5% glycerol in starter 1 and 0% glycerol in starter 2 compared to the other pigs (Table 8). Final body weight after 5 weeks increased ($P = 0.03$) with increasing inclusion of glycerol. ADG was greater in

pigs fed glycerol in starter 2 diets during week 4 ($P = 0.01$), week 5 ($P = 0.007$), the starter 2 phase ($P = 0.002$) and overall ($P = 0.03$). ADFI increased due to the addition of glycerol in phase 2 diets, in week 4 ($P = 0.04$), week 5 ($P = 0.001$), starter 2 phase ($P = 0.003$), and overall ($P = 0.02$). Feed efficiency improved with glycerol supplementation during the starter 2 phase ($P = 0.04$).

Glycerol supplementation did not change serum glycerol concentration during the starter 1 phase (Table 9). Glycerol supplementation had no effect on serum glucose, total protein, albumin, bilirubin, creatine phosphokinase, globulin, or AST. In the starter 2 phase, supplementation of glycerol resulted in a linear increase of serum glycerol concentration during the starter 2 phase ($P < 0.0001$), an increased urea nitrogen ($P = 0.002$), a decreased creatinine ($P = 0.02$), and an increased ratio of blood urea nitrogen to creatinine ($P = 0.0002$). Glycerol supplementation in the starter 2 phase had a quadratic effect on urea nitrogen ($P = 0.0008$), as the 5% level was lower (4.7mg/dl) compared to the 0 and 10% (6.86 and 7.43 mg/dl respectively), and cholesterol ($P = 0.04$), as the value for 5% was higher (92.21 mg/dl) than for the 0% and 10% levels (79.14 and 84.64 mg/dl, respectively). Glycerol supplementation in the starter 2 phase linearly decreased in creatinine ($P = 0.02$) as glycerol levels increased (Table 9).

Experiment 4

Supplementation of glycerol in starter 1 diets linearly increased ($P = 0.05$) pig body weight when measured on week 2 and 3 of the study (Table 10). ADG increased linearly during week 2 ($P = 0.02$) and the starter 1 period ($P = 0.01$) with increasing levels of glycerol. ADFI increased linearly during week 2 ($P = 0.05$) and the starter 1 phase ($P = 0.04$)

as the level of glycerol in the diet increased. G:F was not impacted by glycerol supplementation. Supplementation of 20% lactose compared to 10% lactose in the starter 1 diet had no effect on ADG, ADFI, and G:F. Glycerol supplementation (10%) to diets that had 10% lactose resulted in heavier pig body weights at week 2 ($P = 0.01$), greater ADG during week 1, 2, and the prestarter period ($P < 0.03$), and improved G:F during week 1, 2, and the starter 1 period ($P < 0.04$). There were no differences in growth performance during the starter 2 period, which was after glycerol was removed from the diet.

Serum glycerol linearly ($P = 0.03$) increased as glycerol levels increased in starter 1 of pigs fed glycerol. Glycerol supplementation in starter 1 had no effect on glucose, urea nitrogen, total protein, albumin, alkaline phosphatase, ALT, AST, cholesterol, calcium, phosphorus, sodium, potassium, chloride, albumin, globulin, bun:creatinine ratio, or creatine phosphokinase. Glycerol supplementation decreased creatinine ($P = 0.0004$) and bilirubin ($P = 0.02$) as dietary glycerol levels increased.

Discussion

One of the main objectives of this study was to evaluate the diet manufacturing characteristics of glycerol. The preliminary experiment mixing mash feed demonstrated that diets could be pelleted at glycerol levels of 6 to 8%, so we evaluated pig performance at 0, 5, and 10%. Flowability is an important consideration in the feed milling process. Poor flowability can result in bridging of feed which is one of the three major causes of an out of feed event in grower-finisher facilities (Brumm, 2008). Finisher feed was used in the feed manufacturing studies. Finisher feed does not contain as many poor flow ingredients that are in nursery feed and the level of glycerol we tested was up to 5% in the diet. In Experiment 1,

we observed a linear improvement in flowability as glycerol level increased in mash diets. These results are consistent with research by Groesbeck et al. (2007) who reported the addition of glycerol at levels up to 8% and increased flowability as glycerol levels increased. During the pig performance trials, glycerol did not flow well through the feeders at levels above 5%. The feed flow problem is consistent with Cerrate et al. (2006) who reported problems with poultry diets at 10% glycerol.

The improvement in pellet mill efficiency and pellet durability index as glycerol levels increased (0, 2.5, and 5 %, respectively), are consistent with the results reported by Groesbeck et al. (2008). With an increase in pellet mill efficiency, feed is passed through the pellet mill at a higher rate due to less friction between the pellet die. The benefit to the feed mill is lower electrical costs. Pellet durability improved when glycerol was added. Higher pellet durability is beneficial because it decreases feed wastage, decreases selective feeding, and improves palatability (Behnke, 2001). Pellet durability was also increased when comparing conditioning temperatures of 74°C and 85°C used when pelleting the control diet, which is reported in Briggs et al. (1999). Pellet durability was nearly identical for the diets with 5% glycerol that were pelleted using a conditioning temperature of 74°C compared to the diet with 0% glycerol and a conditioning temperature of 85°C (81.3 and 79.5, respectively). The results suggest the addition of glycerol lower the conditioning temperature without sacrificing pellet quality. Decreased conditioning temperatures would also lower energy costs. The decreases in hot pellet temperature and delta temperature as a result of the addition of glycerol are consistent with Groesbeck et al. (2008).

In Experiment 4, glycerol was applied to the mash diet before pelleting. The production rates were relatively similar for the different levels of glycerol as they were held constant throughout the treatments; however, production rates relative to energy use by the pellet mill motor were greater as the level of glycerol in the diet increased (0, 2.5, 5, 7.5, and 10 %, respectively). Figure 3 shows electrical consumption of the pellet mill motor as impacted by glycerol level in the diet. Electrical energy consumption was reduced by approximately 50% when 7.5 to 10% of glycerol was included compared to 0% glycerol.

Previous studies have shown that feeding glycerol in nursery diets can increase ADG and ADFI without affecting G:F (Groesbeck et al., 2008), while other studies show that glycerol has no effect on the ADG, ADFI, or G:F (Lammers et al., 2008). In Experiment 3, there was an increase in ADG and ADFI in the starter 2 phase but not in the starter 1 phase. Improvements in ADG appeared to be related in part to increased ADFI when glycerol was added to phase 2 diets. In contrast, in Experiment 4, glycerol improved ADG and ADFI in the starter 1 phase at levels up to 10% in the diet.

In Experiment 4 there was also a second control diet in the experimental design which was included to meet two objectives. First, this allowed us to determine the effect of lactose level in the diet (10 vs. 20%) independent of glycerol. Second, it allowed for the comparison of 0 and 10% glycerol supplementation independent of the level of lactose in the diet. Supplementation of 20% lactose compared to 10% lactose in the starter 1 diet had no effect on ADG, ADFI, and G:F. This was surprising as lactose is traditionally included at high levels in the first diet after weaning to ease the transition from sow milk to a solid feed. It would also suggest that any effects of glycerol supplementation were due to glycerol and not

due to the reduction in lactose associated with increasing levels of glycerol. Glycerol supplementation (10%) to diets that had 10% lactose resulted in heavier pig body weights at week 2. There was no effect of glycerol supplementation in phase 1 diets during the starter two phase or the overall nursery period.

Prior studies in other species have shown that ingesting glycerol can increase serum glycerol. Schott et al. (2002) fed glycerol in the water of horses and reported an increase in serum glycerol. Koenigsberg et al. (1995) supplemented human subjects with glycerol in the water and observed increases in serum glycerol. However, Lammers et al (2008) found no increase in serum glycerol when glycerol was supplemented in the diet. In Experiment 3, serum glycerol did not increase in the starter 1 diet when glycerol was fed at 5%. During the starter 2 phase, there was a linear increase in serum glycerol with increasing levels of glycerol in the diet. In Experiment 4, there was linear increase in serum glycerol concentrations when glycerol was supplemented at increasing levels in the starter 1 diet. A plateau appeared to have been reached when glycerol supplementation increased from 7.5% to 10% in the starter 1 diet of Experiment 4 which may indicate a threshold for glycerol in the diet. Bergner and Kijora (1993) reported that as glycerol levels (25.3 and 32.3% in the diet) were increased in the diets of rats, more free glycerol was excreted in the urine. Kijora et al. (1995) found that feeding pigs increasing levels of glycerol (up to 30% in the diet) increased levels of glycerol in the urine.

Glycerol supplementation in swine diets has not been previously reported to affect serum chemistry (Lammers et al. 2008). In Experiment 3, urea nitrogen was impacted in a quadratic manner with the lowest level of BUN at the 5% level, and there was no effect on

urea nitrogen in Experiment 4. The ratio of blood urea nitrogen to creatinine was increased due to glycerol supplementation. The only value that was consistent between both trials was creatinine. In both Experiment 3 and 4 there was a linear decrease in creatinine as glycerol values increased. According to the Merck Veterinary Manual (2009), the range for creatinine in a pig should be between 0.9 and 2.3 mg/dl. In Experiment 3, all of the values for creatinine were below 0.9 mg/dl. In Experiment 4, only the pigs fed 7.5 and 10% glycerol were below 0.9 mg/dl. A decrease in creatinine as glycerol levels increased could be the result of decreased water consumption in the animals that were fed glycerol (Merck 2009). In Experiment 4, two comparisons were completed between pigs fed 20% lactose and 10% lactose and then a comparison between pigs fed 10% lactose with no glycerol added and 10% lactose with 10% glycerol added. There were no differences in serum chemistry with these comparisons.

Supplementation of glycerol to finisher diets in Experiments 1 and 2 improved pellet mill efficiency, pellet durability, and flowability. Application of glycerol before pelleting may improve pelleting efficiency, as it resulted in a 50% reduction in energy use during the pelleting process. Supplementation of glycerol to starter 2 diets in Experiment 3 improved pig performance when added at 5% and 10%; the greatest response was observed at 10%. Although no effect of 5% glycerol supplementation to starter 1 diets on pig performance was observed in Experiment 3, clear effects were noted in Experiment 4. Pig performance was linearly improved with the supplementation of glycerol to starter 2 diets, showing the best results for the highest level (10%) studied. This affect appeared to be independent from lactose levels that were replaced by the glycerol. When glycerol was supplemented in the

diet, it increased serum glycerol at levels above 5% and results on serum chemistry were not consistent. Overall these studies indicate that glycerol can be an asset in feedmilling by improving pellet durability, flowability, pellet mill efficiency, hot pellet temperature, and delta temperature. In nursery diets, it improves ADG, and ADFI at levels up to 10%. Overall data indicated that glycerol can be added in the diets at levels up to 5% and result in improved feedmill performance and pig performance.

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Table 1. Composition of the experimental diets (Experiment 1 and 2), as fed basis

	Control	2.5 % Glycerol	Glycerol
Ingredient			
Corn	82.49	79.72	76.94
Soybean meal (48.5% CP)	11.62	11.87	12.13
Poultry Fat	3.00	3.00	3.00
Crude Glycerol ¹	0	2.50	5.00
Monocalcium phosphate, 21% P	1.04	1.06	1.09
Limestone	1.06	1.05	1.0402
Salt	0.40	0.40	0.40
L-lysine HCl	0.23	0.23	0.23
Mineral premix ²	0.10	0.10	0.10
Threonine	0.03	0.04	0.04
Vitamin Premix ³	0.02	0.02	0.02
Calculated nutrient composition, %			
Crude protein	12.57	12.49	12.41
Calcium	0.65	0.65	0.65
Total phosphorus	0.50	0.50	0.50
Available phosphorus	0.31	0.32	0.32
Lysine	0.75	0.75	0.75
Methionine	0.23	0.23	0.22
Tryptophan	0.13	0.13	0.13
Threonine	0.49	0.49	0.49

¹ contained 86.95% total glycerol, 9.22% moisture, 1.26% sodium, and 1.86% chloride

² Supplied per kg of complete diet: 110 mg of copper as copper sulfate, 198 mg of iodine as ethylenediamine dihydroiodide, 198 mg of iron as ferrous sulfate, 26.4 mg of manganese as manganous oxide, 198.4 mg of selenium as sodium selenite, and 110 mg of zinc as zinc sulfate.

³ Supplied per kg of complete diet: 90,719IU of vitamin A as vitamin a acetate in cross-linked beadlet (preserved with ethoxyquin), 13,636IU of vitamin D₃ as vitamin A acetate with vitamin D₃ cross linked beadlet (preserved with Ethoxyquin), 273IU of vitamin E, 17.6mg of vitamin B₁₂, 3.09g of riboflavin in spray dried riboflavin, 17.64g of niacin, 12.35g of d-pantothenic acid in calcium pantothenate, 1.76g of menadione in menadione sodium bisulfate complex, and 44mg of biotin in spray dried biotin.

Table 2. Composition of the experimental Phase 1 diets (Experiment 3), as fed basis

	Control	Glycerol
Ingredient		
Corn	41.25	41.25
Soybean meal (48.5% CP)	18.26	18.26
Lactose	20.00	15.00
Crude glycerol ¹	0	5.75
Salt	0.28	0.10
Water	0.57	0
Fish meal, menhaden	5.00	5.00
Whey protein concentrate	5.00	5.00
Blood plasma	3.00	3.00
Poultry fat	2.00	2.00
Monocalcium phosphate, 21% P	1.58	1.58
Blood cells	1.50	1.50
Limestone	0.55	0.55
Vitamin-mineral mix	0.40	0.40
Zinc oxide	0.34	0.34
L-lysine HCl	0.14	0.14
DL-methionine	0.07	0.07
Threonine	0.07	0.07
Calculated nutrient composition, %		
Crude protein	21.21	21.21
Calcium	0.80	0.80
Total phosphorus	0.75	0.75
Available phosphorus	0.60	0.60
Lysine	1.50	1.50
Methionine	0.42	0.42
Threonine	0.97	0.97
Tryptophan	0.27	0.27

¹ contained 86.95% total glycerol, 9.22% moisture, 1.26% sodium, and 1.86% chloride

² Supplied per kg of complete diet: 6,112IU of vitamin A, 66 IU of vitamin D-3 as D-activated animal sterol, 33IU of vitamin E, 1.7 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 326mg of choline as choline chloride, 29mg of niacin, 17mg of d-pantothenic acid as calcium pantothenate, 5.1mg of riboflavin, 1mg of pyridoxine as pyridoxine HCl, 1mg thiamine as thiamine mononitrate, 0.02mg of vitamin B₁₂, 1.1mg of folic acid, 0.15mg of d-biotin, 32,971mg Zn as ZnO, 110mg Fe as FeSO₄, 9mg Cu as CuSO₄, 21mg Mn as MnSO₄, 0.25mg I as ethylenediamine dihydriodide, and 0.15mg Se as Na₂SeO₃.

Table 3. Composition of the experimental Phase 2 diets (Experiment 3), as fed basis

	Glycerol, %		
	0	5	10
Ingredient, %			
Corn	64.66	58.48	52.28
Soybean meal (47.5% CP)	29.38	29.95	30.52
Crude glycerol ¹	0	5.75	11.5
Poultry fat	2.00	2.00	2.00
Monocalcium phosphate, 21% P	1.65	1.71	1.76
Limestone	0.91	0.88	0.86
Salt	0.45	0.27	0.10
Vitamin-mineral premix ²	0.40	0.40	0.40
L-lysine	0.31	0.30	0.30
Threonine	0.11	0.12	0.13
DL-methionine	0.05	0.06	0.06
Copper sulfate	0.09	0.09	0.09
Calculated nutrient composition, %			
Crude protein	20.20	20.10	19.9
Calcium	0.75	0.75	0.75
Total phosphorus	0.70	0.70	0.70
Available phosphorus	0.47	0.47	0.48
Lysine	1.30	1.30	1.30
Methionine	0.37	0.37	0.37
Threonine	0.84	0.84	0.84
Tryptophan	0.23	0.23	0.23

¹ contained 86.95% total glycerol, 9.22% moisture, 1.26% sodium, and 1.86% chloride

² Supplied per kg of complete diet: 6,112IU of vitamin A, 661IU of vitamin D-3 as D-activated animal sterol, 33IU of vitamin E, 1.7mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 326mg of choline as choline chloride, 29mg of niacin, 17mg of d-pantothenic acid as calcium pantothenate, 5.1mg of riboflavin, 1mg of pyridoxine as pyridoxine•HCl, 1mg thiamine as thiamine mononitrate, 0.02mg of vitamin B₁₂, 1.1mg of folic acid, 0.15mg of d-biotin, 32,971mg Zn as ZnO, 110mg Fe as FeSO₄, 9 mg Cu as CuSO₄, 21 mg Mn as MnSO₄, 0.25 mg I as ethylenediamine dihydriodide, and 0.15 mg Se as Na₂SeO₃.

Table 4. Composition of the experimental Phase 1 diets (Experiment 4), as fed basis

Ingredient, %	Glycerol, %					Negative Control ¹
	0	2.5	5	7.5	10	
Corn	40.53	40.53	40.53	40.53	40.53	52.90
Soybean meal (47.5% CP)	21.28	21.28	21.28	21.28	21.28	20.13
Crude glycerol ²	0	2.88	5.75	8.63	11.5	0
Lactose	20.00	17.50	15.00	12.5	10.00	10.00
Salt	0.46	0.37	0.28	0.19	0.10	0.46
Water	1.14	0.85	0.57	0.28	0	0
Fish meal, menhaden	5.00	5.00	5.00	5.00	5.00	5.00
Blood plasma	3.00	3.00	3.00	3.00	3.00	3.00
Whey protein concentrate	2.00	2.00	2.00	2.00	2.00	2.00
Poultry fat	2.00	2.00	2.00	2.00	2.00	2.00
Blood cells	1.50	1.50	1.50	1.50	1.50	1.50
Monocalcium phosphate, 21% P	1.50	1.50	1.50	1.50	1.50	1.39
Limestone	0.56	0.56	0.56	0.56	0.56	0.61
Vitamin-mineral premix ³	0.40	0.40	0.40	0.40	0.40	0.40
Zinc oxide	0.34	0.34	0.34	0.34	0.34	0.34
L-lysine	0.15	0.15	0.145	0.15	0.15	0.15
Threonine	0.09	0.09	0.086	0.09	0.09	0.07
DL-methionine	0.07	0.07	0.067	0.07	0.07	0.05
Calculated nutrient composition, %						
Crude protein	21.60	21.60	21.60	21.60	21.60	22.00
Calcium	0.80	0.80	0.80	0.80	0.80	0.80
Total phosphorus	0.75	0.75	0.75	0.75	0.75	0.75
Available phosphorus	0.59	0.59	0.59	0.59	0.59	0.58
Lysine	1.50	1.50	1.50	1.50	1.50	1.50
Methionine	0.42	0.42	0.42	0.42	0.42	0.42
Threonine	0.97	0.97	0.97	0.97	0.97	0.97
Tryptophan	0.27	0.27	0.27	0.27	0.27	0.27

¹ The negative control diet was formulated to contain 10% lactose without glycerol

² contained 86.95% total glycerol, 9.22% moisture, 1.26% sodium, and 1.86% chloride

³ Supplied per kg of complete diet: 6,112 IU of vitamin A, 661 IU of vitamin D-3 as D-activated animal sterol, 33 IU of vitamin E, 1.7 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 326 mg of choline as choline chloride, 29 mg of niacin, 17 mg of d-pantothenic acid as calcium pantothenate, 5.1 mg of riboflavin, 1 mg of pyridoxine as pyridoxine•HCl, 1 mg thiamine as thiamine mononitrate, 0.02 mg of vitamin B₁₂, 1.1 mg of folic acid, 0.15 mg of d-biotin, 32,971 mg Zn as ZnO, 110mg Fe as FeSO₄, 9 mgCu as CuSO₄, 21 mg Mn as MnSO₄, 0.25 mg I as ethylenediamine dihydriodide, and 0.15 mg Se as Na₂SeO₃.

Table 5. Composition of the experimental Phase 2 diet (Experiment 4), as fed basis

Ingredient, %	
Corn	64.66
Soybean meal (47.5% CP)	29.38
Crude glycerol ¹	0
Poultry fat	2.00
Monocalcium phosphate, 21% P	1.65
Limestone	0.91
Salt	0.45
Vitamin-mineral premix ²	0.40
L-lysine	0.31
Threonine	0.11
DL-methionine	0.05
Copper sulfate	0.09
Calculated nutrient composition, %	
Crude protein	20.20
Calcium	0.75
Total phosphorus	0.70
Available phosphorus	0.47
Lysine	1.30
Methionine	0.37
Threonine	0.84
Tryptophan	0.23

¹ contained 86.95% total glycerol, 9.22% moisture, 1.26% sodium, and 1.86% chloride

² Supplied per kg of complete diet: 6,112 IU of vitamin A, 661 IU of vitamin D-3 as D-activated animal sterol, 33 IU of vitamin E, 1.7 mg of vitamin K as menadione dimethylpyrimidinol bisulfate, 326 mg of choline as choline chloride, 29 mg of niacin, 17 mg of d-pantothenic acid as calcium pantothenate, 5.1 mg of riboflavin, 1 mg of pyridoxine as pyridoxine•HCl, 1 mg thiamine as thiamine mononitrate, 0.02 mg of vitamin B₁₂, 1.1 mg of folic acid, 0.15 mg of d-biotin, 32,971 mg Zn as ZnO, 110 mg Fe as FeSO₄, 9 mg Cu as CuSO₄, 21 mg Mn as MnSO₄, 0.25 mg I as ethylenediamine dihydriodide, and 0.15 mg Se as Na₂SeO₃.

Table 6. Effect of glycerol inclusion on flow ability in a mash diet¹ (Experiment 1)

	Glycerol inclusion level			P-Values			
	0%	2.5%	5%	SEM	P<F	lin	quad
Disc size ² (mm)	26.53	25.2	23.73	0.61	0.08	0.03	0.93

¹Each value represents a mean of 3 replicates; linear and quadratic contrast of glycerol supplemented in the diet

²Diameter of the smallest hole that the feed was able to flow through

Table 7. Effect of glycerol supplementation on feedmill characteristics¹ (Experiment 1)

	Glycerol inclusion level				P-Values				
	0% 74°C	0% 85°C	2.5% 74°C	5% 74°C	SEM	P<F	lin	quad	165vs185 ²
Production rate, kg/hr	854.3	868.5	875.0	882.7	21.46	0.18	0.09	0.59	0.34
Horsepower	8.54	7.92	6.30	6.94	0.46	0.1	0.11	0.1	0.38
Pellet Mill Efficiency (Kg/HP)	100.30	109.88	120.16	127.68	2.97	0.01	0.005	0.21	0.06
Pellet durability index, %	55.67	79.50	69.60	81.30	1.96	0.004	0.0001	0.7	0.0002
Conditioning temperature, °C	74.28	85.18	74.00	74.1	0.54	0.64	0.64	0.44	<.0001
Hot pellet temperature, °C	79.07	85.74	77.78	77.41	0.8	0.1	0.05	0.43	<.0001
Delta temperature, °C	4.79	0.57	3.78	3.31	0.68	0.04	0.02	0.27	<.0001
Mashed Moisture %	13.71	13.68	13.87	14.26	0.05	0.11	0.07	0.23	0.66
Conditioned Mashed Moisture %	17.29	18.07	18.13	18.11	0.29	0.12	0.08	0.23	0.11
Pellet Moisture %	14.30	14.63	15.04	15.10	0.59	0.62	0.41	0.67	0.71

¹Each value represents a mean of 3 replicates; linear and quadratic contrast of glycerol inclusion in diets pelleted at 74°C

² Represents the contrast of 74°C vs 85°C within the control diets without glycerol

Table 8. Growth performance of nursery pigs fed crude glycerol to supply 0 or 5% glycerol in starter phase 1 diets and 0, 5, or 10% glycerol in starter phase 2 diets¹ (Experiment 3)

	0% glycerol in starter 1			5% glycerol in starter 1			SEM	P values ²		
	Glycerol in starter 2			Glycerol in starter 2						
	0%	5%	10%	0%	5%	10%		Start 1	Start 2	S1 x S2
Body weight, kg										
Initial	6.95	6.87	6.91	6.89	6.92	6.93	0.03	0.979	0.525	0.179
Week 1	8.08	7.59	7.91	7.85	7.90	8.08	0.17	0.559	0.267	0.256
Week 2	10.50	9.93	9.93	9.75	10.43	10.21	0.25	0.947	0.908	0.039
Week 3	13.31	12.73	12.75	12.43	12.75	13.32	0.33	0.727	0.681	0.107
Week 4	16.91	16.28	16.74	15.65	16.91	17.56	0.44	0.856	0.149	0.093
Week 5	20.32	20.05	20.32	18.50	20.66	21.50	0.53	0.980	0.027	0.020
Average daily gain, g/d										
Week 1	162	103	142	137	140	164	23	0.545	0.326	0.382
Week 2	346	334	288	272	363	305	29	0.687	0.188	0.166
Week 3	401	400	403	383	331	444	35	0.589	0.274	0.308
Week 4	513	508	570	460	594	606	31	0.369	0.010	0.091
Week 5	569	629	597	475	625	656	35	0.661	0.007	0.108
Starter 1	254	219	215	204	251	235	18	0.950	0.856	0.062
Starter 2	491	506	520	438	511	564	20	0.946	0.002	0.066
Overall	394	388	394	342	404	429	16	0.972	0.029	0.025
Average daily feed intake, g/d										
Week 1	226	184	192	209	207	220	13	0.299	0.251	0.184
Week 2	406	352	376	328	400	372	24	0.578	0.923	0.043
Week 3	630	611	622	561	595	649	27	0.384	0.305	0.223
Week 4	737	826	745	706	829	875	42	0.332	0.036	0.157
Week 5	921	1075	1081	821	1067	1078	58	0.444	0.001	0.645
Starter 1	316	268	284	268	304	296	15	0.996	0.911	0.029
Starter 2	751	826	806	690	819	857	32	0.831	0.003	0.241
Overall	571	596	591	516	606	626	23	0.879	0.019	0.169
Gain/feed, g/kg										
Week 1	716	537	746	643	662	721	81	0.892	0.261	0.449
Week 2	854	948	781	819	909	812	58	0.762	0.079	0.792
Week 3	637	651	646	673	557	690	48	0.900	0.381	0.282
Week 4	656	614	766	653	726	699	37	0.635	0.103	0.062
Week 5	617	590	589	578	583	612	34	0.791	0.905	0.664
Starter 1	804	810	766	760	824	788	31	0.910	0.386	0.522
Starter 2	656	613	652	634	626	664	14	0.913	0.044	0.400
Overall	693	652	674	661	668	689	13	0.973	0.244	0.133

¹ Each value represents the mean of 7 pens with 3 pigs per pen

² Probability values for the effects of glycerol in the phase 1 diets (Starter 1; fed for 2 weeks), phase 2 diets (Starter 2; fed for 3 weeks), and their interaction (S1 x S2).

Table 9. Serum chemistry of nursery pigs fed crude glycerol to supply 0 or 5% glycerol in starter phase 1 diets and 0, 5, or 10% glycerol in starter phase 2 diets (Experiment 3)

	Starter 1		p-values		Starter 2			p-values	
	0%	5%	SEM	P<F	0%	5%	10%	SEM	P<F
Glycerol (mg/dl)	32.37	32.52	1.85	0.95	29.48	32.78	35.07	2.26	<.0001
Glucose (mg/dl)	113.33	111.66	2.51	0.95	111.14	114.48	111.86	3.08	0.73
Urea Nitrogen(mg/dl) ²	5.84	6.83	0.44	0.62	6.86	4.71	7.43	0.53	0.002
Creatinine (mg/dl) ¹	0.63	0.67	0.02	0.23	0.67	0.68	0.61	0.02	0.02
Total Protein (g/dl)	4.85	4.91	0.08	0.44	4.94	4.81	4.89	0.10	0.69
Albumin (g/dl)	2.85	2.91	0.08	0.64	2.92	2.80	2.91	0.10	0.59
Bilirubin (mg/dl)	0.11	0.10	0.01	0.34	0.11	0.11	0.11	0.01	0.98
Alkaline Phosphatase (U/L)	254.5	233.0	13.61	0.42	215.9	255.3	260.1	16.69	0.13
ALT SGPT (U/L)	41.68	41.05	2.37	0.07	45.07	41.22	37.79	2.90	0.21
AST SGOT (U/L)	43.36	45.17	3.32	0.05	49.14	41.79	41.86	4.08	0.35
Cholesterol (mg/dl) ³	88.64	82.02	3.33	0.72	79.14	92.21	84.64	4.08	0.09
Calcium (mg/dl)	10.52	10.56	0.12	0.23	10.51	10.69	10.41	0.15	0.41
Phosphorus (mg/dl)	10.52	10.34	0.19	0.09	10.19	10.53	10.57	0.24	0.47
Sodium (mEq/L)	146.41	146.72	0.53	0.17	146.64	147.13	145.93	0.66	0.44
Potassium (mEq/L)	6.23	6.33	0.16	0.14	6.38	6.32	6.16	0.19	0.71
Chloride (mEq/L)	103.52	104.49	0.32	0.20	103.57	103.80	104.64	0.40	0.14
Buncreatine (mg/dl) ²	9.27	10.45	0.66	0.83	10.36	6.93	12.29	0.81	0.002
CPK (U/L)	622	802	101.02	0.42	816	647	672	123.86	0.58

¹linear $P \leq 0.05$

²quadratic $p < 0.001$

³quadratic $p \leq 0.05$

Table 10. Effect of glycerol inclusion rate in Starter I nursery diets on pig performance (Experiment 4)¹

	Glycerol inclusion, %					NC ³	SEM	P values ²			
	0	2.5	5	7.5	10			Lin	Qua d	C1	C2
Body Weight, kg											
Initial	6.67	6.68	6.67	6.66	6.68	6.76	0.04	0.94	0.91	0.18	0.21
Week 1	7.71	7.81	7.88	7.80	8.06	7.74	0.12	0.07	0.72	0.85	0.08
Week 2	9.49	9.54	10.13	9.72	10.39	9.45	0.25	0.01	0.76	0.90	0.01
Week 3	11.95	11.93	12.71	12.17	12.74	11.96	0.30	0.05	0.90	0.98	0.07
Week 4	14.19	14.38	15.00	14.54	14.86	14.48	0.45	0.29	0.65	0.65	0.56
Average daily gain, kg/d											
Week 1	0.15	0.16	0.17	0.16	0.20	0.14	0.02	0.08	0.76	0.77	0.03
Week 2	0.25	0.25	0.32	0.27	0.33	0.24	0.02	0.02	0.87	0.76	0.02
Week 3	0.35	0.34	0.37	0.36	0.34	0.36	0.03	0.86	0.61	0.86	0.58
Week 4	0.32	0.35	0.33	0.34	0.30	0.36	0.04	0.71	0.54	0.46	0.30
Starter 1	0.20	0.20	0.25	0.22	0.27	0.19	0.02	0.01	0.79	0.73	0.01
Starter 2	0.34	0.35	0.35	0.35	0.32	0.36	0.03	0.72	0.47	0.53	0.28
Overall	0.27	0.27	0.30	0.28	0.29	0.28	0.02	0.30	0.62	0.75	0.51
Average daily feed intake, kg/d											
Week 1	0.20	0.20	0.20	0.21	0.23	0.21	0.02	0.35	0.44	0.85	0.48
Week 2	0.33	0.34	0.44	0.43	0.39	0.35	0.03	0.05	0.11	0.78	0.32
Week 3	0.49	0.47	0.56	0.51	0.51	0.49	0.03	0.40	0.46	0.95	0.58
Week 4	0.67	0.70	0.74	0.71	0.76	0.73	0.04	0.18	0.82	0.31	0.64
Starter 1	0.37	0.27	0.32	0.32	0.31	0.28	0.02	0.04	0.28	0.75	0.26
Starter 2	0.58	0.59	0.65	0.61	0.64	0.61	0.03	0.23	0.61	0.51	0.58
Overall	0.42	0.43	0.49	0.46	0.46	0.44	0.02	0.12	0.33	0.51	0.49
Gain/feed, kg/kg											
Week 1	0.73	0.81	0.83	0.77	0.87	0.65	0.05	0.16	0.68	0.29	0.01
Week 2	0.76	0.75	0.77	0.64	0.89	0.69	0.07	0.51	0.17	0.43	0.04
Week 3	0.72	0.73	0.67	0.69	0.66	0.74	0.05	0.29	0.98	0.72	0.19
Week 4	0.47	0.50	0.45	0.47	0.40	0.48	0.05	0.34	0.58	0.85	0.26
Starter 1	0.74	0.76	0.78	0.68	0.87	0.68	0.05	0.27	0.25	0.35	0.01
Starter 2	0.57	0.59	0.54	0.57	0.51	0.59	0.04	0.23	0.65	0.77	0.13
Overall	0.63	0.65	0.62	0.62	0.63	0.62	0.03	0.73	0.86	0.77	0.81

¹ Each value represents the mean of 8 pens with 3 pigs per pen

² Lin is the linear effect of glycerol supplementation; Quad is the quadratic effect of glycerol supplementation; C1 is the contrast between 20% lactose and 10% lactose in diets without glycerol (0% glycerol vs. NC); C2 is the contrast between 0 and 10% glycerol in diets with 10% lactose (10% glycerol vs. NC).

³ NC is the negative control diet containing 10% lactose and 0% glycerol

Table 11. Effect of glycerol inclusion on serum chemistry in starter 1 diets (Experiment 4)

	Glycerol Inclusion						P values ²	
	0%	2.50%	5%	7.50%	10%	NC	SEM	lin
Glycerol (mg/dl)	7.1	7.5	31.1	128.2	97.0	6.9	23.20	0.003
Glucose (mg/dl)	111.38	108.50	123.00	118.75	111.00	110.13	5.86	0.61
Urea Nitrogen(mg/dl)	10.00	8.88	13.88	6.25	9.38	8.25	1.81	0.5
Creatinine (mg/dl)	0.95	0.94	0.93	0.78	0.71	0.94	0.05	0.004
Total Protein (g/dl)	4.76	4.84	4.78	4.55	4.55	4.71	0.13	0.09
Albumin (g/dl)	3.23	3.16	3.13	2.96	3.01	3.06	0.12	0.10
Bilirubin (mg/dl)	0.22	0.16	0.15	0.10	0.11	0.13	0.04	0.03
Alkaline Phosphatase (U/L)	400.6	386.5	365.0	386.9	428.6	392.8	30.42	0.56
ALT SGPT (U/L)	26.00	28.00	24.88	23.38	27.38	31.13	2.88	0.83
AST SGOT (U/L)	45.75	43.25	39.25	31.75	47.00	40.13	9.02	0.75
Cholesterol (mg/dl)	84.13	73.88	90.50	83.50	75.25	75.75	7.40	0.73
Calcium (mg/dl)	10.94	10.80	10.79	10.85	10.76	10.65	0.18	0.59
Phosphorus (mg/dl)	10.16	9.98	10.09	10.54	10.26	9.94	0.27	0.37
Sodium (mEq/L)	142.63	142.38	146.63	142.25	142.25	143.25	1.95	0.89
Potassium (mEq/L)	6.09	6.14	6.04	6.01	6.35	6.06	0.22	0.57
Chloride (mEq/L)	102.75	102.88	105.88	102.13	100.75	103.38	1.49	0.32
Buncreatine (mg/dl)	10.38	9.50	14.88	8.25	11.63	8.88	1.81	0.83
CPK (U/L)	842	671	1012	550	1336	469	354.49	0.44

¹ Lin is the linear effect of glycerol supplementation

²NC is the negative control diet containing 10% lactose and 0% glycerol

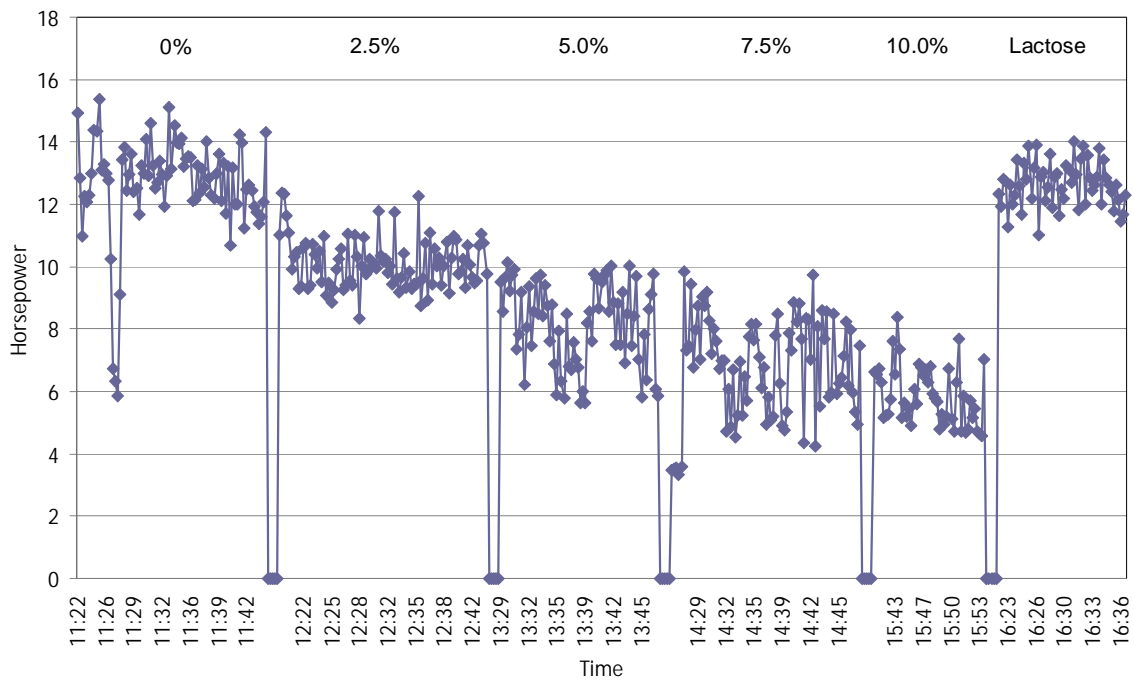


Figure 3. Effect of glycerol inclusion (0, 2.5, 5, 7.5, and 10%) in starter I diets on electrical consumption (horsepower) of the main pellet mill motor (Experiment 4).

¹Refers to the negative control treatment that contained 0% glycerol and 10% lactose whereas the control treatment contained 0% glycerol and 20% lactose