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EFFECTS OF CRUDE GLYCERIN ON FEEDLOT PERFORMANCE, CARCASS CHARACTERISTCS, HORMONE AND METABOLITE CONCENTRATIONS, AND THE FATTY ACID PROFILE IN MARKET LAMBS

A Thesis

Submitted to the Faculty

of

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Ashley F. Musselman

In Partial Fulfillment of the

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This thesis is dedicated to the family and friends that have encouraged and supported me throughout my life and this journey. To my parents, David and Rhonda, you have always provided me with your insight while encouraging me to make my own decisions and stand behind them. Thank you for your unconditional love and support. To my grandparents, you have allowed me to thoroughly enjoy my college career; I feel I will never be able to repay you for you love and support, both emotionally and financially. Thank you!

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LIST OF ABBREVIATIONS

ABBREVIATIONS	DEFINITION
ADG	average daily gain
BW	body weight
°C	degree celcius
CLA	conjugated linoleic acid
cm	centimeter
CON	control treatment
d	day
DM	dry matter
DMI	dry matter intake
FA	fatty acid
g	gram
G:F	gain to feed ratio
h	hour
HCW	hot carcass weight
kg	kilogram
LM	longissimus muscle
min	minute
mg	milligram
MUFA	monounsaturated fatty acid
PUFA	polyunsaturated fatty acid
SAS	statistical analysis software
SEM	standard error of the mean
SFA	saturated fatty acid
Wt	weight

ABSTRACT

Musselman, Ashley F., Purdue University, August 2008. Effects of Crude Glycerin on Feedlot Performance, Carcass Characteristics, Hormone and Metabolite Concentrations, and the Fatty Acid Profile in Market Lambs. Major Professor: Clint P. Rusk.

The objective of this study was to determine the effects of feeding crude glycerin on feedlot performance, carcass characteristics, hormone and metabolite concentrations, and the fatty acid profile in market lambs. Forty-eight crossbred lambs (31.9 ± 5.4 kg; 24 ewes, 24 wethers; 1 ewe and 1 wether/pen; 6 pens/treatment) were blocked by weight and randomly assigned to one of four dietary treatments: 1) 25% dried distiller's grains with solubles (CON), 2) control diet with 15% glycerin (15GLYC), 3) control diet with 30% glycerin (30GLYC), and 4) control diet with 45% glycerin (45GLYC). Crude glycerin (approximately 90% glycerin) replaced corn in the diet on a one to one basis. Weights were taken every 21 days to monitor body weight (BW) change. Lambs were harvested when wethers reached an approximate 12th rib fat depth of 0.51 centimeters. Lambs fed CON and 15GLYC treatments had greater dry matter intake (DMI; $P < 0.001$) and fewer days on feed ($P < 0.001$) compared with both the 30GLYC and 45GLYC treatments. Lambs fed the CON and 15GLYC treatments had greater average daily gain (ADG; $P < 0.001$) and lower gain to feed ratio (G:F; $P < 0.004$) compared with lambs fed the 30GLYC and 45GLYC treatments. No differences were detected in final body weight ($P = 0.74$), hot carcass weight (HCW; $P = 0.78$), longissimus muscle (LM) area ($P = 0.45$),

body wall thickness ($P = 0.41$), flank streaking ($P = 0.24$), or leg score ($P = 0.21$) due to dietary treatment. Lambs fed CON and 15GLYC diets also had greater dressing percentage ($P = 0.01$), 12th rib fat depth ($P = 0.002$), numerical yield grade (YG; $P = 0.003$), and tended to have greater LM ether extract ($P = 0.09$) compared with lambs fed both the 30GLYC and 45GLYC diets. Lambs fed the CON treatment tended to have greater ($P = 0.06$) serum glucose concentration than the lambs fed the 15GLYC, 30GLYC, and 45GLYC treatments. Circulating serum insulin concentrations were greatest ($P = 0.001$) at 4 h postprandial and least at 0 h. Lambs on the CON and 15GLYC treatments had the greatest insulin concentrations at 4h; however, lambs on the 30GLYC and 45GLYC treatments had greatest concentrations at 2h. Lambs on the 15GLYC treatment tended ($P = 0.08$) to have greater insulin concentrations. The β-hydroxybutyrate levels were significant by treatment ($P = .001$), time ($P = 0.001$), and treatment by time ($P = 0.02$). Lambs on the CON and 45GLYC treatments had lower β-hydroxybutyrate concentrations than the lambs on both the 15GLYC and 30GLYC treatments. Total fatty acid concentrations of s.c. adipose ($P = 0.44$) and LM ($P = 0.33$) did not differ between treatments, however, lambs fed the CON and 15GLYC treatments had greater total intake fatty acid concentrations ($P = 0.001$) compared with lambs fed the 30GLYC and 45GLYC treatments. Lambs fed the CON and 15GLYC diets had greater s.c. adipose tissue concentrations of oleic acid (18:1n-9*trans*-11; $P = 0.01$), linoleic acid (18:2n-6*cis*; $P = 0.001$), and conjugated linoleic acid (*trans*-10, *cis*-12; $P = 0.003$) compared with lambs on the 30GLYC and 45GLYC treatments. Longissimus muscle (LM) fatty acid concentrations of conjugated linoleic acid ($P = 0.25$) showed no difference between treatments; however, lambs fed the CON and 15GLYC diets had

greater concentrations of oleic acid (18:1n-9*trans*-11; $P = 0.001$) and PUFA ($P = 0.001$) compared with lambs fed the 30GLYC and 45GLYC diets. These results imply the 30GLYC and 45GLYC treatments had ill effects on feedlot performance, carcass characteristics, hormone and metabolite concentrations, and fatty acid profile of market lambs. Based on the findings from this study, the 15GLYC treatment is the most beneficial glycerin treatment for producers to utilize based on cost and results from the feedlot performance, carcass characteristics, hormone and metabolite concentrations, and the fatty acid profile of market lambs.

Keywords: Feedlot, Glycerin, Lamb

CHAPTER 1

LITERATURE REVIEW

Introduction

Proper animal nutrition is essential to good animal health. Determining what to feed can be difficult for livestock producers of the wide variety of feedstuffs to choose from, and because the nutritional needs of the animal change throughout its life. The amount of feed and the type of feedstuffs utilized depend on the stage of life the animal is in, and on the purpose the animal is intended for.

Feedstuffs provide energy for several bodily functions including: maintenance, growth, reproduction, lactation, and work (Center for Agricultural and Environmental Research and Training [CAERT], 2002). The lambs in this study were fed-out for approximately four months, with the primary energy requirement being dedicated to growth of the lambs on trial. Feedstuffs can be expensive; therefore, more studies are needed to test the efficiency and effectiveness of alternative feeds.

Due to the increased interest in ethanol and biodiesel fuel production in the Eastern Corn Belt region (IL, IN, KY, MI, MN, OH, and WI), and the increasing price of corn, there is a need to determine the proper use of glycerin, one of the major co-products of biodiesel production. Currently, the price of corn is about 8 cents per pound. If the ethanol and biodiesel industries continue to thrive, and the demand for corn remains high, glycerin has the potential to be a suitable energy-dense ingredient for ruminant diets.

According to Klare (2005), the proper use of oil is a controversial subject, which can lead to conflict. There have been numerous debates over the implications of the United States' dependence on foreign oil; therefore, alternative fuels have become a

major priority for federal and state leaders. As of August 2007, there were 127 ethanol biorefineries completed in the United States (U.S.) and another 78 under construction (Renewable Fuels Association [RFA], 2005).

According to the RFA (2005), ethanol production continues to consume an increasing percentage of the nation's corn crop – amounting to about 20% in 2006. The corn acreage utilized to produce ethanol is expected to increase about 10% in 2007, to 86 million acres. Although high corn prices are great for grain producers, the high prices create a hardship for livestock producers. Therefore, glycerin could serve as an energy source to meet the nutritional requirement of ruminant diets and potentially, be a cheaper, yet effective, alternative feed source.

According to CAERT (2002), animal growth requires mostly energy from high levels of carbohydrates and fats in the animal's diet and smaller amounts of other nutrients. Lambs are ruminant animals which gives them the unique ability to digest certain feedstuffs, especially roughages, efficiently. A basic understanding of the ruminant's digestive tract is essential for making intelligent feeding decisions.

One of the major challenges for the U.S. Sheep Industry is consumer demand for lamb (Schroeder, Jerrick, Jones, and Spaeth, 2001). Purcell (1989) stated that in the 1970s and 1980s, the per capita consumption of lamb decreased significantly as consumer income increased. U.S. policy makers enacted programs in 2000 to reverse the declining demand for lamb, including earmarking $5 million annually to develop and promote lamb products, which is part of a $100 million multi-year effort to help revitalize the lamb industry (American Sheep Industry Association, 2000). When Glickman (2000) announced the $100 million plan, he also stated that by funding production

improvements, increasing promotion efforts, and helping to improve animal health, our assistance plan boosts the long-term development and growth of lamb and sheep farming in the U.S.

A literature review was completed using professional journals, magazines, books, research articles, abstracts, reports, electronic media and databases. The following terms were searched to find articles relevant to this study: ethanol, ethanol by-products, biodiesel, glycerin, feed additives, energy feed sources, ruminant animals, ruminant nutrition, and distiller grains. A combination of terms was also used to find additional articles.

Ruminant Digestive System

According to Visser (2005), the ruminant digestive tract has three preliminary compartments ahead of the true stomach, or abomasum. These compartments are the reticulum, rumen, and omasum. The rumen and reticulum are not completely separated, but have different functions.

Plant tissues contain about 75 percent carbohydrates of one type or another, which provide the primary energy source for both the rumen organisms and the host animal. In ruminant animals, the major portion of all carbohydrates, including the complex carbohydrates such as cellulose and hemi-cellulose, is digested by bacteria in the rumen. The main end-products of carbohydrate digestion are volatile fatty acids. Of these, acetic acid forms the major proportion, followed in declining order by propionic, butyric, and valeric acids. The volatile fatty acids are absorbed into the bloodstream through the rumen wall, and constitute 66 to 75% of the energy derived from feed. Carbohydrates,

such as sugars and starches, which escape ruminal digestion, are broken down in the abomasum and the end-products are absorbed through the small intestine.

Nutrient Requirements for Ruminant Animals

The five nutrient groups required by livestock include: energy, protein, vitamins, minerals, and water (Gillespie, 2002). These groups are all required in proper amounts to support the life of the animal. Good nutrition can increase feed efficiency and rate-of-gain in animals. If the nutritional needs of the animal are not met, the animal will not grow as fast and could possibly even die. Carbohydrates, fats, and oils are good sources of energy for animal diets. Cereal grains like corn, wheat, sorghum, barley, rye, and oats are a few examples of those good energy sources. However, corn prices are extremely high due to the increase in ethanol and biodiesel manufacturing. By using by-products of this manufacturing, such as glycerin and distillers dried grains, livestock producers can reduce the cost of energy in their rations and still meet the nutrient requirements.

Animal growth requires mostly energy and smaller amounts of other nutrients. Carbohydrates should make up about 75 percent of the animal's diet (CAERT, 2002). Growth of ruminant animals implies the overall increase in muscles, bones, internal organs, and other body parts. Using too much of any one nutrient in an animal's diet is wasteful and could prove harmful to the animal's health. For this reason, this study sought to determine the optimum level of dietary glycerin to maximize the growth of market lambs.

Offering by-products, such as crude glycerin, for use as animal feed can be an economical and environmentally friendly way for manufacturers to reduce waste and cut

costs. Instead of paying to have glycerin removed, manufactures can make an additional profit by selling glycerin to livestock producers. According to Crickenberger and Carawan (1996), producers should consider the following factors to determine if a material is appropriate for a particular animal feeding situation: types and proportions of by-products generated, variability in moisture and nutrient content, storability of the material, handling characteristics, potential for the presence of physical contaminants, and potential for development of molds and related mycotoxins.

Biodiesel / Ethanol

Tyson (2000) reported that biodiesel is produced through a process in which organically derived fats and oils are chemically reacted with a short chain alcohol, such as methanol, and a catalyst to produce biodiesel and the glycerin co-product. Biodiesel is a renewable fuel that is made from a variety of natural fats and oils (Sheehan, Camobreco, Duffield, Graboski and Shapouri, 1998) including: soybean or Canola oils, animal fats, waste vegetable oils, or microalgae oils.

The Iowa Department of Agriculture and Land Stewardship Office of Renewable Fuels and Co-products reported that ethanol production consumes the grain's starch, while a variety of highly valuable feed co-products are produced from the remaining protein, fiber, vitamins and minerals in dry mill corn processing. At dry mill ethanol refineries, which make up the majority of ethanol production, most feed is dried and sold as Distillers Dried Grains with Solubles (DDGS). According to the Renewable Fuels Association website, approximately 20-25% of the feed is shipped wet to local producers,

which is great for the manufacturer because it reduces energy input, lowers transportation costs, and provides another market for the livestock producers.

The Renewable Fuels Association also states that a modern dry-mill ethanol refinery produces approximately 2.8 gallons of ethanol and more than 17 pounds of distiller's grains from a single bushel of corn. In 2006, ethanol biorefineries produced approximately 12 million metric tons of distillers grains (Renewable Fuels Assoc., 2005). On August 8, 2005, President Bush signed the Energy Policy Act of 2005 (H.R. 6) into law. This legislation set a nationwide renewable fuels standard (RFS) that will result in the use of more than 7.5 billion gallons of ethanol and biodiesel by 2012. Under the RFS, a small percentage of our nation's fuel supply will be provided by renewable, domestic fuels including ethanol and biodiesel. These fuels will provide a positive roadmap for reduced consumer fuel prices, increased energy security, and growth in rural America. The RFS resulted from years of negotiations between the ethanol industry, oil industry, federal government, state interests, environmentalists, agriculturalists and consumers. These groups worked together to determine the best way to encourage a greater supply from the renewable fuel industry to help meet our nation's energy needs (RFA, 2005).

The production of biodiesel grew from 500,000 gallons in 1999 to 75 million gallons in 2005 and 250 million gallons in 2006 (National Biodiesel Board, 2007). Although ethanol production can come from crops other than corn, ethanol production consumed about 20% of the nation's corn crop in 2006. U.S. corn acreage is expected to increase about 10% in 2007 to 86 million acres. Agricultural experts foresee a limit to corn's biofuel contribution at about 12–15 billion gallons of ethanol, which is about 10% of the gasoline demand in the U.S. (RFA, 2005).

Glycerin

Glycerin, as defined by Westerman (1997), is also known as glycerine or crude glycerin. Glycerin is a colorless, thick liquid that can be dissolved into water or alcohol, but not oils. It is produced in small quantities by alcohol fermentation. Glycerin is a natural, sweet tasting liquid that is a byproduct of biodiesel production. Therefore, it can be an attractive feed additive for ruminant animals (Anonymous, 1995).

The United States Department of Agriculture (USDA) (2007) found that crude glycerin provides an energy source that is equal to or exceeds the energy available from corn. According to Hess (n.d.), disposal of the crude glycerin product is an important consideration for manufacturers of biodiesel. Hess (n.d.) found that the crude glycerin product does not satisfy the legal requirements for pharmaceutical use because crude glycerin has a purity level of about 85 percent, meaning it also contains small amounts of salt, methanol and free fatty acids (Kerr and Dozier, 2007). The process of purifying crude glycerin for pharmaceutical use is costly. However, when glycerin is refined to 99 percent purity, it can be used for pharmaceutical, food, drink, cosmetic and toiletry products (Kerr and Dozier, 2007).

Glycerin is already being used in soaps and some food products, but the amount of glycerin needed for such products is far less than the abundant supply that will result from increased biofuel production. Urbanchuck (2006) estimated that within the next ten years there will be 1.4 billion pounds of glycerin and as much as 200 million pounds in 2007 alone. Currently, glycerin is about 20 to 50 cents-per-pound; however, the price could drop to 5 cents per pound as the co-product becomes more readily available. Selling by-products will produce additional revenue for biofuel manufacturers. If the

co-product is offered as a less expensive feed source than traditional feeds, then buying and utilizing glycerin could save livestock producers money and provide exceptional nutrition that leads to good animal performance.

According to Hess (n.d.), there are no legal restrictions regarding animal species or the amount of glycerin that may be fed to an animal. Thus, marketing crude glycerin as a feed additive for livestock rations is one way to add value to this by-product. Donkin and Doane (2007) found that when feeding sixty lactating Holstein cows diets of 0, 5, 10, and 15% glycerin on a dry matter (DM) basis, there was no difference in milk production, feed intake, milk composition and body condition score among treatments.

According to Chung, Rico, Martinez, Cassidy, Noirot, Ames, and Varga (2007), when dry glycerin was fed at 250 g/d as a top dressing to dairy cows, there was no ill affect on average feed intake, milk yield, blood metabolites, and serum insulin concentrations. Simon, Bergner, and Schwabe (1996) fed broiler chickens glycerin to replace 10% of the energy source, such as corn, in the total ration DM without having negative effects on feed intake, weight gain, and feed conversion ratio. Pyatt et al. (2007) found that when adding glycerin to replace 10% of the corn in the finishing diets of Angus-cross steers, animals fed the glycerin diet had higher average daily gains, and improved feed efficiency.

The main route of glycerol clearance from the rumen is the formation of propionic acid (Kijora, Bergner, Götz, Bartelt, Szakacs, and Sommer, 1997). Glycerin use has a lot of potential. For example, DeFrain, Hippen, Kalscheur, and Jardon (2004) found that glycerin can lessen the symptoms of ketosis when delivered as an oral drench. Glycerin added to the diet eliminates the need for restraining cows for drenching, while delivering

a glucogenic substrate. This then alleviates the fatty liver-ketosis complex, and improves the lactation performance.

Johnson (1955) reported that glycerin was used successfully for therapy of ketotic ewes and cows, and that glycerin drenching is remarkably effective in the treatment of sleeping sickness or pregnancy toxemia in ewes. Ketosis is a metabolic disorder that can result in sheep and cattle. The buildup of significant levels of ketones (a by-product of fat metabolism) in blood plasma is most commonly reported in dairy cows that are kept indoors during the winter months and are fed high concentrate diets. If there is too much mobilization of ketone, it can become toxic for the animal. Schröder and Südekum (1999) fed sheep 48, 78, 131, or 185 g/d of glycerin in a low-starch, concentrate diet and found either no effect or positive effects on digestibility of organic matter, starch, and cell-wall components. However, when these researchers fed the same levels of glycerin in high-starch concentrate diets, they found a decrease in cell-wall digestibility but, no effect on the digestion of organic matter or starch. Schröder and Südekum also found that feeding glycerin decreased the acetate:propionate ratio and stimulated water intake, both of which benefit transition dairy cows.

Johns (1953) and Garton (1961) reported that when crude glycerin was added to sheep rumen contents the formation of propionic acid resulted. Because Hobson and Mann were able to isolate glycerol-fermenting bacteria, *Selemonas* from the rumen contents of sheep in 1961 these results were not surprising.

Glycerin ferments in the rumen before being converted into large amounts of propionate, which is then absorbed through the rumen wall. Some glycerin is also converted to lactate, while the remainder goes to the liver and is changed into glucose.

The major metabolic pathway where glucose is converted to energy has two stages. Glycolysis occurs under aerobic conditions and results in the production of pyruvate. Pyruvate is then transported into the mitosol and oxidized into carbon dioxide and water for further energy production (McDonald, Edwards, Greenhalgh and Morgan, 2002). Therefore, ruminants may be able to utilize the starch from grains and the energy from glycerin in a similar manner.

Hess (n.d.) points out that although studies have shown that it is possible for glycerin to be utilized by ruminants; the previous studies have focused on the use of glycerin in the absence of other fermentable substances. Therefore, researchers are limited when using previous glycerin findings because the potential effects of glycerin on other feed components were not addressed.

Potential Issues with Glycerin

One growth study fed 5% and 10% glycerin to pigs from weaning to market weight as a partial replacement for corn (Wallaces Farmer, 2007). The results showed equal growth performance between the glycerin treatment and the more conventional corn treatment. However, the researchers found that in the diet with the higher percentage of glycerin, the feed did not flow well in a self-feeder because the mix became sticky. Therefore, using a large percentage of crude glycerin in a ration is not practical for swine producers to work with.

Another issue found with the use of glycerol as a feed additive is that methanol, an ingredient used to make biodiesel, is present in small amounts in glycerin. Methanol can have negative side effects, such as blindness, when it is included in animal diets.

According to Methanol Institute (2007), methanol is an alcohol that can be used as an alternative fuel or as a gasoline additive. Methanol is less volatile than gasoline. When blended with gasoline, methanol lowers the carbon monoxide emissions but increases hydrocarbon emissions, and is poisonous to humans and animals if ingested.

Distiller's Grains

According to Dhuyvetter, Kastens, and Boland (2005), distiller's grains are recognized as highly nutritious animal feed ingredients that are highly concentrated in protein, minerals, fat and fiber. Distiller's grains are unique in that they are the only fermented feed ingredient from the dry mill fuel or beverage ethanol process. Distiller's grains are separated during the distillation phase of ethanol production. The solid remains are a by-product of ethanol production, which are currently being marketed as a livestock feed called distillers grains with solubles (DGS). Distiller's grains can be wet (WDGS) or dried (DDGS). Dried distiller's grains with soluble are a beneficial source of nutrition for mixed rations, and provide essential protein and fat for ruminant diets. However, the amount of DDGS a producer should feed is limited because of their high protein and fat content. In 2006, more than 85% of distiller's grains produced in the U.S. were fed to ruminant animals (dairy and beef cattle), 9% to swine and 3% to poultry (Renewable Fuels Assoc., 2005).

Summary

The increase of biodiesel and ethanol production has caused corn prices to rise, and could lead to a surplus of glycerin, which could serve as a corn-supplement and help both biodiesel and ethanol plants, as well as livestock producers. Glycerin is an energy

source that can meet the needs of a ruminant animal's diet at certain percentages of the ration.

Little research has been conducted or published on the effects of including crude glycerin in the diets of market lambs. Such studies have been directed more toward dairy, beef, and swine; which exist in greater numbers and have a higher economic impact in the U.S. than sheep. The lamb industry, however, focuses on higher-valued cuts, and targets the Northeastern states and the West due to the number of immigrants in those areas. Despite low meat prices, a reduced need for wool, and labor shortages, the demand for sheep has remained steady (Jones, 2004). In other efforts to stay competitive, sheep producers have a goal to produce uniform, safe, nutritious, and lean lamb (Shackelford, Leymaster, Wheeler, and Koohmaraie).

Food producers, processors, and manufacturers are lowering the fat content of animal products in order to produce red meat products that meet dietary guidelines (Soloman, Lynch, Ono, and Paroczay, 1990). The content of saturated fatty acids in livestock tissues can be decreased by using alternative feeding systems (Skelley et al., 1973).

The researchers hypothesized that decreased fat content, due to crude glycerin replacing varying amounts of corn in the diet, would decrease the amount of linoleic acid biohydrogenation intermediates deposited in the longissimus muscle (LM) and subcutaneous (s.c.) adipose tissue, or marbling. The objectives were to determine if adding crude glycerin to replace corn would have an effect on the feedlot performance, carcass characteristics, hormone and metabolite concentrations, and fatty acid profile of market lambs.

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CHAPTER 2

Effects of crude glycerin on feedlot performance, carcass characteristics, hormone and metabolite concentrations, and the fatty acid profile in market lambs.

Assumptions and Limitations

Three assumptions were made during this study.

- The lambs were being fed at approximately 8 a.m. every morning.
- Each pair of lambs was fed the appropriate amount of the correct treatment.
- Glycerin's digestibility is equal to that of corn.

A few limitations were recognized within this study.

- Data from this experiment may be limited to locations with similar availabilities, such as access to feed sources and biodiesel manufacturers.

Introduction

The drastic increase in ethanol and biodiesel production has led to elevated prices of traditional feedstuffs; leaving livestock producers searching for alternative feeds to lower production costs, while maintaining animal performance. Production of biodiesel in the U.S. over the next decade is expected to yield an estimated 1.4 billion pounds of glycerin. Although glycerol is not priced to be used economically, increased production could lead to a surplus causing a dramatic decrease in the price. With the increase in corn prices, glycerol has the potential to be a cost-effective alternative energy feed resource for livestock.

In ruminant animals, glycerol can be rapidly converted to propionic acid and readily absorbed through the rumen wall (Kijora et al., 1997). Of the primary volatile fatty acids,

propionate is the only one which is directly gluconeogenic. Feeding glycerol to dairy cows at 250 g/d as a top dressing increased ruminal propionate and subsequently increased circulatory glucose concentration in cattle (Chung et al., 2007). Similarly, total organic matter digestibility was not influenced due to 48, 78, 131, or 185 g/d of glycerol in diets containing low levels of starch (Schröder and Südekum, 1999).

Schröder and Südekum (1999) reported that feeding glycerol decreased the acetate:propionate ratio and stimulated water intake, both of which were beneficial to transition dairy cows. When the acetate to propionate ratio decreases, the energy retention in the animal increases (Wolin, 1960). Johns (1953) reported that adding glycerol to sheep rumen contents resulted in the formation of propionic acid. DeFrain et al. (2004) reported that substitution of corn with glycerol resulted in similar plasma glucose concentrations in dairy cattle, suggesting that glycerol has the potential to act as an energy substitute for ruminant animals. The main route of glycerol clearance from the rumen is the formation of propionic acid (Kijora et al., 1997), which is then absorbed through the rumen wall. Glycerol is also converted to lactate, while the remainder goes to the liver and is changed into glucose. The major metabolic pathway where glucose is converted to energy has two stages. Glycolysis occurs under aerobic conditions and results in the production of energy. Ruminants may be able to utilize the starch from grains and the energy from glycerin in a similar manner. Therefore, our objective was to determine if crude glycerin may act as a viable energy substitute in rations of finishing lambs.

Materials and Methods

Animals and diets. Animal care and use was conducted in accordance with a protocol approved by the Purdue University Animal Care and Use Committee. The research was conducted from May through September, 2007 at the Purdue University Sheep Unit. Forty-eight crossbred lambs (31.9 ± 5.4 kg; 24 ewes, 24 wethers) were blocked by weight and randomly assigned (1 ewe and 1 wether/pen; 6 pens/treatment) to one of four isocaloric dietary treatments (Table 1): 1) 25% dried distiller's grains with solubles (CON), 2) control diet with 15% glycerin (15GLYC), 3) control diet with 30% glycerin (30GLYC), and 4) control diet with 45% glycerin (45GLYC). Crude glycerin (approximately 90% glycerin) replaced corn in the diet on a one to one basis. Huls et al. (2006) reported that DDGS can be used in lamb rations as the primary protein source without causing ill effect on growth and carcass characteristics, and Zelinsky et al. (2006) stated that DDGS offer a palatable feed ingredient for lamb diets.

Before the start of the study, lambs were ear-tagged, dewormed, and vaccinated with Clostridium Perfringens Type C & D with Tetanus Toxoid (CD/T). For a complete list of initial weights and treatment groups see Appendix A. Throughout the duration of the trial, lambs were housed in 1.83 x 1.83 m pens, fed once daily in the morning, and had free access to water. Lambs were adapted to their diet over a 2 week preliminary period. By mistake, one of the 45GLYC pens was assigned ewe/ewe in a pen; therefore, the data for that pen was removed. Dry matter intake was calculated on a pen average. Daily intake of fatty acids (Table 7) was calculated by dry matter intake per day (g/d) multiplied by the percentage of the specific fatty acid in the sample.

Feedlot performance and carcass characteristics. Weights of the lambs were collected every 21 days to monitor BW change. Although all lambs were finished-out, wether lambs were harvested when their back fat thickness reached approximately 0.51 cm; the estimated 12th rib fat depth was determined by visual appraisal and palpation handling of each wether. At this point, the paired ewe lamb was removed from the study and returned to the Purdue University flock.

Wether lambs were harvested at the Purdue University Meat Laboratory where HCW was recorded. Carcass data were collected (Appendix C) after a 24 h chill, and the following measurements collected by trained Purdue University personnel: 1) flank streaking scores; 2) LM area (taken by tracing the loin muscle at the 12th rib); and 3) leg conformation scores. A 12th rib muscle sample was stored for later ether extract analysis (see Appendix H).

Sampling and laboratory analysis. Dietary samples were collected periodically throughout the study. Samples collected for analysis were dried in a forced air oven for 48 h at 60°C for DM, and analyzed for nitrogen content (Leco FP analyzer model 602600, Leco Corp., St. Joseph, MI); NDF and ADF (ANKOM$^{200/220}$ Fiber Analyzer, ANKOM Technology, Fairport, NY).

Longissimus and s.c. adipose tissue samples were obtained 24 h post harvest from the lamb carcasses and were stored at -20°C for fatty acid analysis. Fatty acid analysis on feed samples, LM tissue, and s.c. adipose tissue were analyzed at the USDA-ARS, Northern Great Plains Research Laboratory (Mandan, ND) using an acid catalyst in direct-trans esterification outlined by Kucuk et al. (2001; see Appendix G). Preparation

of the s.c. adipose and LM tissues was outlined by Murrieta et al. (2003) (see Appendix H). Fatty acid concentrations were determined by Gas Chromatography (Model 3800, Varian Inc., Palo Alto, CA) using a 100-m capillary column (Supelco 2560, Supelco, Bellefonte, PA). Helium, the carrier gas, was maintained at a column flow of 1.5 mL/min. The oven temperature was maintained at 120˚C for 2 minutes, increased up to 175˚C at a 6˚C/min interval, and finally brought up to 250˚C at a 10˚C/min interval. The injector temperature was kept at 260˚C while the detector temperature was held at 300˚C. The split ratio for the s.c. adipose tissue was 100:1 and for LM muscle 30:1. Purified fatty acid standards (Sigma-Aldrich, St. Louis, MO; Nu-Check Prep, Elysian, MN, Matreya, Pleasant Gap, PA) were used to identify the individual peaks. The s.c. adipose tissue (Table 8) and the LM tissue (Table 9) fatty acids were measured in mg of fatty acid per g of tissue.

Hormones and metabolites. On day 56 of the study, a preprandial blood sample was collected into vacutubes from all lambs. After the first sample was collected, lambs were given one hour to consume their diets. Postprandial blood samples were then collected every hour for four hours. The blood samples were kept on ice. Upon returning to the lab, samples were placed in a refrigerator and kept at 2-4 °C. All samples were centrifuged, and then serum was collected. All samples were frozen then later analyzed for glucose, insulin, and β-hydroxybutrate concentration.

Preprandial and postprandial plasma samples were analyzed for glucose (Glucose Liqui-UV Hexokinase Procedure No. 1060, Stanbio Laboratory, Boerne, TX; intra- and interassay CV of 7.4 and 8.6, respectively; see Appendix D for protocol). The glucose

determination procedure was modified using a standard calibration curve consisting of 0, 50, 100, 125 and 250 mg of glucose/dL created from dilution of a 1000mg/dL glucose standard. Sample preparation was modified by adding 3 µL of plasma to 300µL of reagent on 350µL, UV-transparent 96-well plates (Becton, Dickinson and Co., Franklin Lakes, NJ) and read on an Opsys MR microplate reader (Dynex Technologies Inc., Chantilly, VA) at 340 nm. Preprandial and postprandial plasma samples were analyzed for insulin using an RIA kit (Coat-A-Count Insulin, Siemens Medical Solutions USA Inc., Malvern, PA; intra- and interassay CV of 4.9 and 2.6%, respectively). β-hydroxybutyrate concentrations were analyzed using the Stanbio β-hydroxybutrate LiquiColor® kit (Procedure No. 2440; see Appendix F). For the β-hydroxybutyrate test, blood samples taken at 0, 2, and 4h were analyzed; this will show a better curve, and be representative of time.

Statistical analyses. Data were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Treatment was tested against the following dependent variables: DMI, ADG, G:F, days on feed, carcass characteristics, hormone and metabolite concentrations, and the fatty acid profiles of market lambs. The model statement included the effect of treatment on the specific variables to calculate the variations from the mean. For insulin data, insulin values were not corrected for standards.

Results and Discussion

Growth performance/feed data. The effects of crude glycerin on feedlot performance of market lambs are presented in Table 2. Lambs fed the CON and 15GLYC treatments had

the greater dry matter intake (DMI; P <0.0001) and fewer days on feed (P <.0001) compared with both the 30GLYC and 45GLYC treatments. Lambs fed the 30GLYC and 45GLYC treatments had reduced DMI due to problems with palatability and texture of those diets. Lambs fed the CON and 15GLYC treatments had greater average daily gain (ADG; P <0.001) and lower gain to feed ratio (G:F) (P <0.0004) compared with lambs fed the 30GLYC and 45GLYC treatments. The lambs on the CON and 15GLYC treatments finished an average of 28 d earlier than those on the 30GLYC treatment and an average of 61 d earlier than the lambs on the 45GLYC treatment. The increased number of days on feed when glycerol is fed at levels greater than 15% could be a significant economic disadvantage because it is costing producers more money to finish out lambs on the higher glycerin treatments. The increase in ADG and G:F observed in the CON and 15GLYC lambs on this study are similar to the results reported when 10% glycerin was added to the finishing diet of Angus-cross steers (Pyatt et al., 2007). Schröder and Südekum (1999) reported no difference in DMI when glycerol was fed at 10% of DM as a replacement for fermentable starch in the diet of dairy cows, which is similar to the results of DMI for the comparison of lambs fed the CON and 15GLYC treatments.

The cost comparison to finish lambs out on each of the four treatments is presented in Table 3. If crude glycerin was sold for the anticipated $0.05/lb, it would be economically beneficially to utilize the glycerin at 15, 30, and 45 percent of the ration, however, producers must also consider if the percent utilized will be practically beneficial. If crude glycerin was sold at $0.10/lb it would be economically friendly to utilize glycerin at 15 and 30 percent of the ration. If crude glycerin was sold for $0.15/lb

it would only be economically beneficial for producers to utilize glycerin at 15 percent of the lamb ration.

Carcass characteristics. The effects of crude glycerin on carcass characteristics of market lambs are presented in Table 4. No differences were detected in final BW (P = 0.74), HCW (P = 0.78), 12th rib LM area (P = 0.45), body wall thickness (P = 0.41), flank streaking (P = 0.24), or leg score (P = 0.21) due to dietary treatment. However, lambs on the CON and 15GLYC treatments had the highest dressing percentage (P = 0.01), back fat thickness (P = 0.002), numerical yield grade (P = 0.003), and tended to have greater LM ether extract (P = 0.09) compared to the 30GLYC and 45GLYC treatments, indicating that lambs fed the CON and 15GLYC treatments were fatter, likely due to increased levels of fat in their diets and increased DMI.

Results from Table 4 show drastic differences in fat depth among the treatments. As previously stated, wethers were harvested when an estimated 12th rib fat depth of .51 cm was reached. However, as shown in Table 5, lambs fed the 30GLYC and 45GLYC treatments did not reach that level. After an end date for the study was determined, many of the lambs from the 30GLYC and 45GLYC treatment had not grown efficiently. Although they were heavy enough, they did not gain enough fat; therefore, they were harvested without reaching the intended .51 cm 12th rib fat depth.

Hormones and metabolites. Glycerol is a gluconeogenic precursor, meaning that it can be converted to propionate in the rumen and subsequently converted to glucose by the liver (McDonald, Edwards, Greenhalgh and Morgan, 2002). Glucose levels were

measured to determine if the dietary treatments altered circulating serum glucose levels by feeding glycerol as opposed to corn, or another feed source.

The effects of crude glycerin on glucose, insulin, and β-hydroxybutrate concentrations of market lambs are reported in Table 6. See Appendix B for blood sample notes. Lambs on the CON treatment tended to have greater ($P = 0.06$) serum glucose concentrations compared with lambs fed the 15GLYC, 30GLYC, and 45GLYC treatments. A comparison of serum glucose and insulin concentrations is shown in Figure 1. The glucose levels were not significant by time ($P = 0.34$).

Insulin tightly regulates cellular glucose uptake, and as glucose levels increase, insulin levels follow by spiking. Postprandial, blood glucose levels increase slightly, and cause the pancreas to release insulin into the bloodstream to utilize the glucose, ultimately lowering blood sugar levels. When lambs are on a high concentrate diet, they may become insensitive to insulin (Trenkle, 1970). Insensitivity to insulin could result in lambs becoming less efficient. Insulin concentrations were significant by time ($P = 0.001$). Insulin concentrations were the greatest for the 4 h samples and the least for the 0 h samples. Lambs on the 15GLYC treatment tended to have greater insulin concentrations when analyzed by treatment ($P = 0.08$).

Glycerol can be converted to a ketone, therefore, β-hydroxybutyrate was measured to determine if there was an increased synthesis of ketones. β-hydroxybutyrate levels were significant by treatment ($P = 0.001$), time ($P = 0.001$), and treatment x time ($P = 0.02$). Lambs on the CON and 45GLYC treatments had lower β-hydroxybutyrate concentrations than the lambs on both the 15GLYC and 30GLYC treatments. The samples from the 0h had lower concentrations compared to the samples from 2h and 4h.

The higher β-hydroxybutyrate levels in lambs on the 15GLYC and 30GLYC treatments indicate there was an increase of dietary glycerol being converted to ketones due to the addition of glycerol in the diets. β-hydroxybutyrate concentrations are used to determine ketosis in dairy cows, the cutoff levels for β-hydroxybutyrate for animals in a negative energy balance ranges from 10 to 15 mg/dL (Overton, 2000). Although the β-hydroxybutyrate levels were statistically significant, the levels are not biologically significant because finishing lambs are not in a negative energy balance.

Subcutaneous adipose tissue. Total fatty acid concentrations in s.c. adipose tissue did not differ ($P = 0.44$) between treatments (Table 8). The lambs fed the CON diet had greater concentrations of capric acid (10:0; $P = 0.04$) and lower concentrations of pentadecanoic acid (15:0; $P = 0.001$), margaric acid (17:0; $P = 0.003$), margaroleic acid (17:1; $P = 0.002$), and arachidic acid (20:0; $P = 0.03$) compared with lambs fed the 15GLYC, 30GLYC, and 45GLYC diets. Lambs fed the CON and 15GYLC diets had greater concentrations of oleic acid (18:1n-9*trans*-11; $P = 0.01$), linoleic acid (18:2n-6*cis*; $P = 0.001$), conjugated linoleic acid (*trans*-10, *cis*-12; $P = 0.003$), heneicosanoic acid (21:0; $P = 0.002$), behenic acid (22:0; $P = 0.01$), and erucic acid (22:1n-9; $P = 0.005$) compared with the lambs on the 30GLYC and 45GLYC diets. Lambs fed the CON diet had greater concentrations of gamma-linolenic acid (18:3n-6; $P = 0.01$), nervonic acid (24:1; $P = 0.001$), and polyunsaturated fatty acids (PUFA; $P = 0.001$) compared with lambs on the 15GLYC, 30GLYC, and 45GLYC diets, which could be a result of the decreased fat in the 15GLYC, 30GLYC, and 45GLYC diets due to the zero fat content of glycerin.

Longissimus muscle tissue. Total fatty acid concentrations did not differ ($P = 0.33$) due to dietary treatments (Table 9). Lambs fed CON and 15GLYC diets had lower concentrations of pentadecanoic acid (15:0; $P = 0.001$), margaric acid (17:0; $P = 0.001$), margaroleic acid (17:1; $P = 0.001$), and eicosadienoic acid (20:2; $P = 0.001$) compared with the lambs on 30GLYC and 45GLYC diets. Lambs fed CON and 15GLYC diets had greater concentrations of oleic acid (18:1n-9*trans*-11; $P = 0.001$), heneicosanoic acid (21:0; $P = 0.001$), and polyunsaturated fatty acids (PUFA; $P = 0.001$) compared with both the 30GLYC and 45GLYC treatment groups. These findings could be a result of decreased DMI for the diets containing glycerin amounts, and the decreased fat in the 15GLYC, 30GLYC, and 45GLYC diets due to the zero fat content of glycerin. The reduction in DMI levels can lead to energy and nutrient deficiencies that are essential for maintaining normal bodily functions. For example, reduced concentrations of linoleic acid could lead to improper growth and development (Conner, 1999), and increased levels of CLA concentrations in the diet has potential health benefits including anti-cancer effects, reduces fat deposits, prevention of diabetes, and enhanced bone formation (Belury, 2002).

Implications

These results imply that adding crude glycerin to the finish lamb diet can be a cost efficient decision for producers. However, the 30GLYC and 45GLYC treatments had ill effects on feedlot performance, carcass characteristics, hormone and metabolite concentrations, and fatty acid profile of market lambs. Based on the findings from this study, the 15GLYC treatment is the most beneficial glycerin treatment for producers to

utilize based on cost and results from the feedlot performance, carcass characteristics, hormone and metabolite concentrations, and the fatty acid profile of market lambs.

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Table 1. Composition of diets fed to finishing market lambs.[1] (DM %)

	CON	15GLYC	30GLYC	45GLYC
Ingredient				
Alfa-grass hay	10.8	10.7	10.6	19.9
Corn	59.9	24.5	8.1	--
DDGS	25.1	25.0	25.2	25.1
Glycerin	--	14.9	30.6	44.7
Soyhulls	--	19.5	17.9	--
Gluten	--	1.2	3.4	6.1
Mineral	3.0	3.0	3.0	3.0
Molasses	1.2	1.2	1.2	1.2
Nutrient				
CP	14.1	14.0	14.2	14.1
TDN	82.3	82.1	82.6	82.2
Fatty Acid Profile				
12:0	0.05	0.03	0.04	0.01
14:0	0.04	0.02	0	0.02
16:0	10.11	7.38	7.06	5.32
18:0	1.57	1.29	1.26	0.89
18:1n-9cis	16.84	12.13	11.91	8.37
18:2n-6cis	40.34	29.49	27.81	19.06
18:3n-3	1.39	1.51	1.42	0.95
22:2	0.19	0.17	0.16	0.07
Total	72.03	53.19	50.73	35.77
Unidentified	1.54	1.19	1.08	1.11
Saturated FA	11.73	8.70	8.36	6.22
MUFA	16.84	12.13	11.91	8.37
PUFA	41.92	31.16	29.38	20.07
TUFA	58.76	43.29	41.29	28.44

[1] Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).

Table 2. Effects of dietary treatment on finish lamb performance.[a]

Item	CON	15GLYC	30GLYC	45GLYC	SEM[b]	P value
Lambs, no.	12	12	12	10		
Initial Wt, kg	29.0	28.7	29.2	30.3	0.57	0.18
Start Wt, kg	33.6	32.8	33.5	33.8	0.79	0.81
End Wt, kg	54.8	53.8	56.4	55.5	1.82	0.74
DMI, kg	2.48^{cd}	2.61^{c}	2.30^{d}	1.89^{e}	0.08	0.001
ADG, kg/d	0.26^{c}	0.25^{c}	0.21^{d}	0.15^{e}	0.02	0.001
Feed efficiency, G:F, kg	0.09^{c}	0.09^{cd}	0.08^{d}	0.07^{e}	0.01	0.004
Days on feed	82.7^{c}	82.5^{e}	110.8^{d}	143.4^{c}	8.80	0.001

[a]Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).

[b]Greatest SE is presented

[c-f]Means within a row lacking a common superscript differ

Table 3. Cost of finishing lamb rations[1] with the inclusion of crude glycerin at varying levels and prices.

	CON	15GLYC	30GLYC	45GLYC
Ave Daily Cost	$0.80			
$0.05/lb glycerin		$0.67	$0.53	$0.46
$0.10/lb glycerin		$0.72	$0.61	$0.55
$0.15/lb glycerin		$0.76	$0.69	$0.65
Cost per Gain (kg)	$3.12			
$0.05/lb glycerin		$2.63	$2.56	$3.03
$0.10/lb glycerin		$2.83	$2.95	$3.62
$0.15/lb glycerin		$2.98	$3.33	$4.28
Total Cost to Finish	$66.16			
$0.05/lb glycerin		$55.28	$58.72	$65.96
$0.10/lb glycerin		$59.40	$67.59	$78.87
$0.15/lb glycerin		$62.70	$76.45	$93.21

[1]Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).

Table 4. Effects of dietary treatment on carcass characteristics of finishing lambs.[a]

Item	CON	15GLYC	30GLYC	45GLYC	SEM[b]	P value
Lambs, no.	6	6	6	5		
Hot carcass Wt, kg	32.4	32.5	31.8	30.9	1.30	0.78
Dressing Percentage	57.7[c]	58.5[c]	55.5[d]	55.3[d]	0.80	0.01
Fat Depth, cm	0.67[c]	0.65[c]	0.38[d]	0.34[d]	0.07	0.002
Ribeye Area, cm^2	57.2	54.7	68.3	46.2	10.00	0.45
Body Wall, cm	2.70	2.70	2.30	2.30	0.25	0.41
Yield Grade	2.96[c]	2.99[c]	1.88[d]	1.84[d]	0.26	0.003
Flank Streaking	20.7	19.8	20.0	19.4	0.50	0.24
Leg Score	13.0	13.0	13.3	12.2	0.40	0.21
Ether Extract % Fat	9.4	7.2	4.7	4.2	1.50	0.09

[a]Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).

[b]Greatest SE is presented

[c,d]Means within a row lacking a common superscript differ

Table 5. Average fat depth and treatment[1] distribution of wethers (n) per slaughter day.

Treatment[1]	57	78	106	120	140	157
CON	.7(2)	.7(2)	.8(1)	.5(1)	--	--
15GLYC	.8(3)	.5(1)	.6(1)	--	.5(1)	--
30GLYC	--	.4(2)	.4(2)	--	.4(1)	.4(1)
45GLYC	--	--	.4(1)	--	.5(1)	.3(3)

[1]Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).

Table 6. Circulating glucose, insulin, and β-hydroxybutyrate concentrations in finishing lambs.[a]

Item	CON	15GLYC	30GLYC	45GLYC	SEM[b]	P value
Glucose, mg/dL	72.5	66.2	59.9	62.6	3.44	0.06
Insulin, ng/ml	20.6	43.4	13.8	9.1	10.19	0.08
B-hydroxybutyrate, mg/dL	0.12[d]	0.18[c]	0.15[c]	0.13[d]	0.01	0.001

[a]Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).

[b]Greatest SE is presented

[c,d]Means within a row lacking a common superscript differ

Table 7. Effects of added crude glycerin on fatty acid intake of finishing lambs.[1]

	Dietary Treatment					P value[3]
Fatty Acid	CON	15GLYC	30GLYC	45GLYC	SEM[2]	Treatment
	Total, g/d of fatty acid intake					
	200.1[a]	169.4[b]	141.8[c]	82.8[d]	18.63	0.001
	g/d of fatty acids					
12:0	0.15[a]	0.10[b]	0.11[b]	0.03[c]	0.01	0.001
14:0	0.12[a]	0.06[b]	0.00[c]	0.04[d]	0.01	0.001
16:0	28.1[a]	23.5[b]	19.7[c]	12.3[d]	2.61	0.001
18:0	4.37[a]	4.10[a]	3.51[b]	2.06[c]	0.42	0.001
18:1n-9*cis*	46.8[a]	38.6[b]	33.3[b]	19.4[c]	4.35	0.001
18:2n-6*cis*	112.1[a]	93.9[b]	77.7[c]	44.1[d]	10.40	0.001
18:3n-3	3.86[b]	4.80[a]	3.97[b]	2.20[c]	0.40	0.001
22:2	0.53[a]	0.55[a]	0.44[b]	0.16[c]	0.05	0.001
Saturated FA	32.6[a]	27.7[b]	23.4[c]	14.4[d]	3.04	0.001
MUFA	46.8[a]	38.6[b]	33.3[c]	19.4[d]	4.35	0.001
PUFA	116.5[a]	99.2[b]	82.2[c]	46.5[d]	10.84	0.001
TUFA	163.3[a]	137.9[b]	115.5[c]	65.8[d]	15.19	0.001
Unidentified	4.27[a]	3.78[b]	3.03[c]	2.57[c]	0.40	0.001

[1]Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).

[2]Greatest SE is presented

[3]Probabilities for overall treatment F-test and for orthogonal contrasts between CON vs. elevated glycerin diets.

[a-d]Means within a row lacking a common superscript differ

Table 8. Effects of increasing levels of crude glycerin on the fatty acid profile of subcutaneous adipose tissue from finishing lambs.[1]

	Dietary Treatment					P value[3]
Fatty Acid	CON	15GLYC	30GLYC	45GLYC	SEM[2]	Treatment
	Total, mg of FA/g adipose tissue					
	639.6	655.6	651.7	587.5	33.40	0.44
	mg of FA/g of adipose tissue					
10:0	0.99[a]	0.84[ab]	0.86[ab]	0.68[b]	0.07	0.04
12:0	0.72	0.61	0.85	0.47	0.16	0.35
14:0	15.27[a]	14.17[ab]	16.26[a]	10.85[b]	1.56	0.10
14:1	0.38	0.41	0.40	0.33	0.06	0.83
15:0	3.75[c]	5.17[b]	6.33[a]	6.08[ab]	0.39	0.003
16:0	127.0[a]	122.6[a]	123.5[a]	100.7[b]	7.53	0.08
16:1	10.89	11.38	12.57	10.93	0.84	0.41
17:0	13.6[c]	19.6[b]	22.7[b]	27.3[a]	1.87	0.003
17:1	4.41[c]	6.02[bc]	7.68[b]	10.68[a]	0.83	0.002
18:0	104.6	110.6	121.2	100.0	10.70	0.50
18:1n-9*trans*-11	74.8[a]	77.8[a]	52.8[b]	22.7[c]	8.81	0.01
18:1n-9*cis*	200.7	201.9	205.0	222.6	12.48	0.56
18:1*cis*-11	7.41	6.84	6.62	6.34	0.77	0.76
18:2n-6*cis*	42.5[a]	27.0[b]	22.9[bc]	18.8[c]	2.35	0.001
20:0	0.43[b]	0.59[b]	0.72[ab]	0.76[a]	0.04	0.03
18:3n-6	0.14[a]	0.01[b]	0.08[ab]	0.02[b]	0.03	0.01
18:3n-3	2.37	2.27	2.12	1.67	0.22	0.14
18:2n-*cis*9,*trans*-11 CLA	3.19	3.90	3.99	2.74	0.53	0.28
18:2n-*trans*-10,*cis*-12 CLA	0.53[a]	0.39[b]	0.24[c]	0.18[c]	0.05	0.003
21:0	0.32[a]	0.23[b]	0.15[c]	0.16[c]	0.03	0.002
22:0	0.27[a]	0.23[a]	0.15[b]	0.17[b]	0.03	0.01
22:1n-9	1.36[a]	1.00[b]	0.90[b]	0.97[b]	0.09	0.005
24:1	0.11[a]	0.02[b]	0.01[b]	0.00[b]	0.01	0.001
22:6n-3	0.46	0.42	0.37	0.32	0.07	0.51
Saturated FA	267.4	275.0	293.0	247.5	17.07	0.29
MUFA	299.6	305.4	286.0	274.6	17.87	0.59
PUFA	49.7[a]	34.9[b]	30.7[bc]	24.4[c]	2.62	0.001
TUFA	349.3	340.3	316.7	299.0	19.77	0.26
Unidentified	22.9	40.4	42.0	41.0	7.65	0.20

[1]Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).

[2]Greatest SE is presented

[3]Probabilities for overall treatment F-test and for orthogonal contrasts between CON vs. elevated glycerin diets.

[a-c]Means within a row lacking a common superscript differ

Table 9. Effects of increasing levels of crude glycerin on the fatty acid profile of longissimus muscle tissue from finishing lambs[1]

	Dietary Treatment					P value[3]
Fatty Acid	CON	15GLYC	30GLYC	45GLYC	SEM[2]	Treatment
	Total, mg of FA/g of loin muscle tissue					
	111.9	118.3	97.9	92.7	11.43	0.33
	mg of FA/g of LM tissue					
10:0	0.14	0.13	0.11	0.10	0.02	0.48
12:0	0.12	0.09	0.07	0.08	0.02	0.14
14:0	2.28	2.14	1.70	1.59	0.30	0.28
14:1	0.02	0.02	0.01	0.00	0.01	0.74
15:0	0.36^{c}	0.50^{bc}	0.59^{b}	0.84^{a}	0.07	0.001
16:0	23.5	24.4	20.1	18.4	2.62	0.30
16:1	1.88	1.90	1.81	1.95	0.22	0.97
17:0	1.32^{c}	2.14^{b}	2.41^{b}	3.46^{a}	0.30	0.001
17:1	0.70^{c}	1.03^{bc}	1.38^{b}	2.26^{a}	0.16	0.001
18:0	14.42^{ab}	17.39^{a}	13.61^{ab}	11.19^{b}	1.73	0.10
18:1n-9*trans*-11	6.98^{a}	7.67^{a}	4.19^{b}	2.01^{c}	0.92	0.001
18:1n-9*cis*	41.2	45.0	38.2	37.5	5.01	0.66
18:1*cis*-11	1.84	1.60	1.40	1.50	0.14	0.13
18:2n-6*cis*	10.80^{a}	8.76^{b}	7.01^{c}	6.03^{c}	0.43	0.001
18:3n-3	0.44	0.48	0.38	0.35	0.05	0.24
18:2n-cis9, trans-11 CLA	0.46	0.62	0.51	0.40	0.08	0.25
21:0	0.10^{a}	0.07^{b}	0.03^{c}	0.01^{d}	0.01	0.001
20:2	0.12^{c}	0.15^{c}	0.22^{b}	0.29^{a}	0.02	0.001
22:0	0.20	0.19	0.18	0.17	0.01	0.27
22:1n-9	2.36	2.24	2.33	2.58	0.14	0.35
20:3n-3	0.11	0.09	0.09	0.10	0.01	0.56
24:1	0.06	0.04	0.05	0.07	0.01	0.34
22:6n-3	0.33	0.32	0.32	0.34	0.03	0.97
Saturated FA	42.8	47.4	39.2	36.2	4.87	0.37
MUFA	55.0	59.5	49.4	47.8	6.12	0.47
PUFA	12.26^{a}	10.41^{b}	8.53^{c}	7.51^{c}	0.48	0.001
TUFA	67.3	69.9	58.0	55.3	6.42	0.28
Unidentified	1.74	0.95	0.74	1.21	0.64	0.66

[1]Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).

[2]Greatest SE is presented

[3]Probabilities for overall treatment F-test and for orthogonal contrasts between CON vs. elevated glycerin diets.

[a-d]Means within a row lacking a common superscript differ

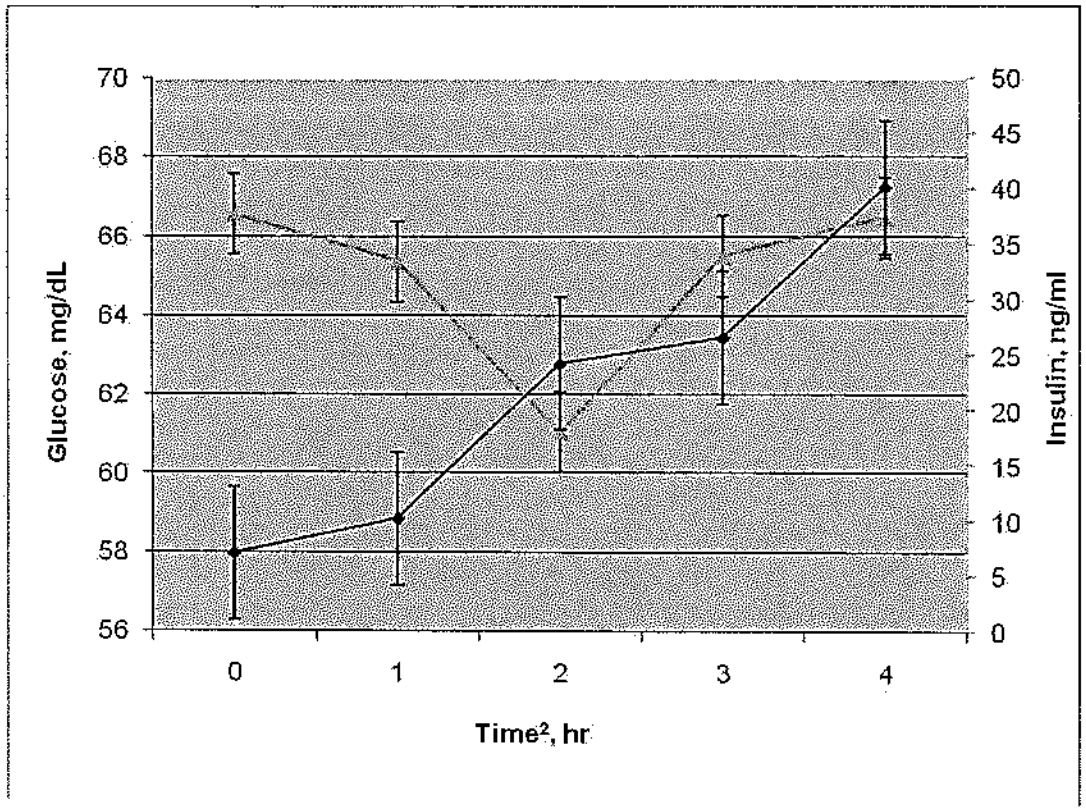

Figure 1. Effects of dietary treatment on serum glucose and insulin concentrations of finishing lambs.[1]

[1]Dietary treatments: corn-based feedlot ration (CON), CON diet with 15% glycerin (approximately 90% glycerol) (15GLYC), CON diet with 30% glycerin added at the expense of corn (30GLYC), and CON diet with 45% glycerin at the expense of corn (45GLYC).
[2]Preprandial (0h) and postprandial (1-4h) samples were taken hourly.
♦Represents insulin concentrations, significant by time ($P = 0.001$)
▲Represents serum glucose concentrations, not significant by time ($P = 0.35$)

CHAPTER 3

Summary and Conclusion

Summary

The increase of biodiesel and ethanol production has caused corn prices to rise, and has lead to a surplus of crude glycerin. Crude glycerin could serve as a corn-supplement and help both biodiesel manufacturers, as well as livestock producers. The experiment was conducted to determine the effects of crude glycerin on feedlot performance, carcass characteristics, hormone and metabolite concentrations, and fatty acid profile of market lambs.

Little research has been conducted and/or published on sheep feeding studies involving crude glycerin. Such studies have been directed more toward dairy, beef, and swine. This is largely due to the fact those species exist in greater numbers and have a higher economic impact in the U.S. compared with sheep. In efforts to stay competitive, sheep producers have a goal to produce uniform, safe, nutritious, and lean lamb (Shackelford et al.,n.d.).

Conclusion

These results imply that adding crude glycerin to the finish lamb diet can be a cost efficient decision for producers. However, the 30GLYC and 45GLYC treatments had ill effects on feedlot performance, carcass characteristics, hormone and metabolite concentrations, and fatty acid profile of market lambs. Based on the findings from this study, the 15GLYC treatment is the most beneficial glycerin treatment for producers to utilize based on cost and results from the feedlot performance, carcass characteristics, hormone and metabolite concentrations, and the fatty acid profile of market lambs.

APPENDICES

Appendix A: Initial Weights and Treatments

Tag #	Weight (day1)	Weight (day2)	Average Weight	Sex	Diet	Pen
103	46	50	48	R	1	1
290	51	53	52	E	1	1
322	48	49	48.5	R	1	2
125	54	51	52.5	E	1	2
242	59	60	59.5	R	1	3
268	63	64	63.5	E	1	3
203	64	64	64	E	1	4
266	74	75	74.5	R	1	4
321	68	69	68.5	E	1	5
299	75	80	77.5	R	1	5
254	74	75	74.5	E	1	6
217	80	84	82	R	1	6
241	51	50	50.5	E	6	31
126	51	50	50.5	R	6	31
127	46	50	48	E	6	32
296	53	57	55	R	6	32
316	59	64	61.5	E	6	33
221	61	64	62.5	R	6	33
264	64	67	65.5	E	6	34
298	70	73	71.5	R	6	34
333	66	67	66.5	E	6	35
663	72	74	73	R	6	35
229	67	70	68.5	E	6	36
682	79	81	80	R	6	36
224	50	51	50.5	R	7	37
277	51	51	51	E	7	37
323	55	54	54.5	E	7	38
233	55	57	56	R	7	38
329	62	61	61.5	E	7	39
313	61	64	62.5	R	7	39
339	64	68	66	E	7	40
336	67	66	66.5	R	7	40
208	70	74	72	R	7	41
259	71	75	73	E	7	41
210	77	80	78.5	R	7	42
234	79	80	79.5	E	7	42
116	50	51	50.5	R	8	43
222	51	51	51	E	6	43
239	55	56	55.5	R	8	44
205	55	57	56	E	6	44
337	62	61	61.5	E	8	45
228	62	62	62	R	8	45
340	66	64	65	E	8	46
278	70	74	72	R	8	46
235	65	70	67.5	E	8	47
251	77	80	78.5	R	8	47
219	71	74	72.5	E	8	48
263	76	81	78.5	R	8	48

Appendix B: Blood Sample Notes

Sheep Study 2007 Blood Sample Notes

Diet	Pen	Tag #	
1	1	103	
1	1	290	
1	2	322	
1	2	125	
1	3	242	
1	3	268	
1	4	203	
1	4	266	
1	5	321	
1	5	299	
1	6	254	
1	6	217	
6	31	241	
6	31	126	
6	32	127	
6	32	296	
6	33	316	
6	33	221	
6	34	264	No 3 hr sample
6	34	298	
6	35	333	
6	35	663	
6	36	229	
6	36	682	
7	37	224	
7	37	277	
7	38	323	
7	38	233	
7	39	329	
7	39	313	
7	40	339	
7	40	336	
7	41	208	
7	41	259	
7	42	210	
7	42	234	
8	43	116	
8	43	222	
8	44	239	
8	44	205	No 2 hr sample
8	45	337	
8	45	228	
8	46	340	
8	46	278	
8	47	235	
8	47	251	2 samples 1 hr
8	48	219	
8	48	263	

Appendix C: Lamb Carcass Evaluation Form

Lamb Evaluation Form

Student Name_____ Number_____ Date_____

Ranges	90-160	45.0-58.0	.05-0.50	1.5-3.3	1.0-5.9			
Avg.	130	52% unshorn 54% shorn	0.25	2.4	2.9			

Animal Number	Comments		Live Wt.	Dressing %	Adj 12th Ribfat Thick.	Ribeye Area	Yield Grade	Maturity	Flank Streaking	Conformation
1		Est								
		Act								
2		Est								
		Act								
3		Est								
		Act								
4		Est								
		Act								
5		Est								
		Act								

USDA Quality Grade Code

Prime^{+} = 22	Choice^{-} = 17	Utility^{+} = 12
Prime^{0} = 21	Good^{+} = 16	Utility^{0} = 11
Prime^{-} = 20	Good^{0} = 15	Utility^{-} = 10
Choice^{+} = 19	Good^{-} = 14	Cull = 09
Choice^{0} = 18		

Appendix D: Glucose Protocol

Glucose Protocol

*Be sure to use only 96-well plates that are readable in UV conditions.

- Put 300 uL of Glucose reagent in each well using multi-channel pipet man.

- Using the 0.1-10 uL pipet, add 3uL of either standard or sample to each well, pipetting up and down 4 or 5 times to mix and be sure all sample is out of tip (make sure to keep plunger down until tip is removed from well to avoid drawing sample back up.

- Once wells are filled place plate in 37°C incubator for 5 minutes.

- After 5 minutes, place plate in reader and read plate (it may be necessary to rerun plate in the reader so that proper mixing occurs).

STANDARDS

250 mg/dL = 100 microliters of stock glucose standard: 300uL D.I. water

125 mg/dL = 100uL stock: 700uL water

100 mg/dL = 100uL stock: 900uL water

50 mg/dL = 100uL stock: 1900uL water

0 mg/dL = 1000uL water

Appendix E: β-hydroxybutyrate Protocol

β-hydroxybutyrate LiquiColor
Stanbio Procedure No. 2440

Standards Concentration: 12.807 mg/dL

Standards:

A) 12.609 mg/dL (1mmol/L): 1 mL 12.807 standard
B) 6.305 mg/dL (0.5mmol/L): 100uL A: 100uL water
C) 3.152 mg/dL (0.25mmol/L): 50uL B: 50uL water
D) 1.576 mg/dL (0.125mmol/L): 50uL C: 50uL water
E) 0 mg/dL (0mmol/L): 100uL water

Procedure:

- Incubate needed amount of Reagent A at 37°C for 3 minutes
- Put 239uL Reagent A into 96-well plate
- Add 7uL sample to wells
- Add 40uL of Reagent B to each well
- Incubate for 5 minutes at 37°C
- Measure OD

Calculation:
B-hydroxybutyrate (mM) =
[OD (5min) Sample / OD (5min) Std] x 1mM x dilution of serum

Appendix F: Fatty Acid Concentration Protocol (Feeds and Forages)

Direct Trans-esterification using an acid catalyst (HCl)

For use on Feeds and Forage

1. Weigh in internal standard (*13:0 or 21:0; 1.0 $CHCl_3$ containing 1mg/mL of methylated internal standard*) into a 16 mm × 125 mm tube with Teflon cap. May need to use a bigger tube depending on size of sample.
2. Dry down under N
3. Weigh sample (*at least 0.200 g but no more than 0.500*)
4. Add 2 mL 1.09 M Methanolic-HCl
    a. Samples must be "swimming" in 1.09 M Methanolic-HCl, therefore, volumes may need to be adjusted.
    b. 0.5 g of forage may require 4 mL of 1.09 M Methanolic-HCl
5. Add 2 mL (*or same volume as in step 4*) of MEOH
6. Heat at 80°C for 1 hr. VORTEX at least every 3 minutes
    a. Watch to make sure MEOH is not boiling off, if so be sure caps are very tight
7. Cool samples to room temperature
8. Add 2 mL (*or same volume as in step 4*) deionized H_20
9. Add 2 mL (*or same volume as in step 4*) Hexane
10. Vortex (*15 seconds*)
11. Centrifuge at 2000 rpm for 3 minutes
12. Transfer top layer (*Hexane layer*) into GC vial that contains a bed of sodium sulfate
13. Analyze on GC

Reagents

1.09 M Methanolic-HCl (500 mL)

1. Add 45.6 mL of concentrated HCl to 459.4 mL MEOH

OR

2. Weigh 54.1 g of HCl and 352.9 g of MEOH

Internal standard (100 mL)

Weigh 100 mg of internal standard (13:0 or 21:0) and add to 100 mL $CHCl_3$ (or 149 g (1.49 g/mL))

Reference

Kucuk, O., B. W. Hess, P. A. Ludden, and D. C. Rule. 2001. Effect of forage:concentrate ratio on ruminal digestion and duodenal flow of fatty acids in ewes. J. Anim. Sci. 79:2233–2240.

Appendix G: Fatty Acid Concentration Protocol (LM muscle and s.c. Adipose)

Direct Trans-esterification using an alkaline catalyst (KOH)

For use on Adipose, Muscle, and Milk

14. Weigh out tissue samples as needed
    a. Muscle (no more than 150 mg)
    b. Adipose (no more than 30 mg)
15. Add 2 mL 0.2 M Methanolic - KOH
16. Vortex
17. Heat at 50°C for 30 min. VORTEX every 3 minutes
18. Add 1 mL saturated aqueous NaCl
19. Add 2 mL hexane containing internal standard
20. Vortex (*15 seconds*)
21. Centrifuge at 3000 rpm for 3 minutes
22. Transfer top layer (*Hexane layer*) into GC vial that contains a bed of sodium sulfate
23. Analyze on GC

Reagents

0.2 M KOH in methanol

1. Weigh 5.61 g of KOH in 500 mL of MEOH (or 359.6 g of MEOH)

Internal standard (500 mL)

1. Weigh 100 mg of internal standard (methyl-tridecanoic acid (13:0) and add to 100 mL hexane (or 65.9 g (0.659 g/mL))

Citation

Murrieta, C. M., B. W. Hess, and D. C. Rule. 2003. Comparison of acidic and alkaline catalysts for preparation of fatty acid methyl esters from ovine muscle with emphasis on conjugated linoleic acid. Meat Science. 65:523-529.

Appendix H: Ether Extract Protocol

FAT EXTRACTION

Supplies: Ethyl ether
Filter papers, 12.5 cm, Whatman No. 1

1. Dry filter papers 4 h or overnight at 105°C. 2 papers folded in thirds and then again in thirds.
2. Weigh filter papers. Let equilibrate with humidity in environment.
3. Weigh 2 to 3 g of sample (less if fat only) onto filter paper and fold up. Record exact weight
4. Dry sample packets same as above.
5. Weigh after drying.
6. Place samples in Soxhlet extraction units – 3 packets on bottom and 3 packets of top of that in extraction unit. Fill bottle with ethyl erther – approx. 500ml, turn on cooling water, plug in electricity. Extract for 6 h.
7. Remove from extraction unit, let packets air dry in hood for approx. 30 min and then place in oven and dry as above.
8. Weigh samples again

$$\frac{(\text{sample} + \text{filter}) - (\text{dry sample} + \text{filter})}{\text{Sample}} * 100 = \% \text{ water}$$

$$\frac{\text{dry sample} - \text{extracted sample}}{\text{Sample}} * 100 = \% \text{ fat}$$