

EFFECTS OF CRUDE GLYCERIN IN FEEDLOT CATTLE

by

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B.S., Texas A&M University, 2004

M.S., Kansas State University, 2007

AN ABSTRACT OF A DISSERTATION

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Department of Animal Sciences and Industry  
College of Agriculture

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## Abstract

Two trials were conducted to evaluate the effects of crude glycerin in feedlot cattle. The objectives of this research were to determine the effects of crude glycerin on animal performance, carcass characteristics, ruminal metabolism, and diet digestibility when fed in steam-flaked corn finishing diets. Trial one utilized crossbred heifers ( $n = 373$ ) fed finishing diets containing 0, 2, 4, 8, 12, or 16% crude glycerin. Feeding heifers crude glycerin at 8% or less of the diet resulted in improvements in body weight gain and feed efficiency. Dry matter intake decreased linearly ( $P < 0.001$ ) when glycerin was included at increasing levels from 0 to 16%. Average daily gains of heifers fed crude glycerin increased when glycerin was fed at 2, 4, or 8% of diet DM, but reductions in ADG were noted when glycerin increased to 12 or 16% (linear,  $P = 0.013$ ; quadratic,  $P = 0.010$ ). Feeding glycerin had a quadratic effect on G:F, and was optimal when fed at 2% of DM ( $P = 0.46$ ). Hot carcass weights increased when glycerin was fed at 2, 4, and 8% of the diet, but decreases in HCW were observed with 12 and 16% crude glycerin (linear,  $P = 0.009$ ; quadratic,  $P = 0.006$ ). Low concentrations of glycerin can be fed without negatively impacting animal performances. Trial two consisted of a  $3 \times 3$  Latin Square and utilized cannulated crossbred steers ( $n = 9$ ) fed finishing diets containing 0, 2, or 4% crude glycerin. Apparent total tract digestibilities of DM, OM, starch, CP, and crude fat were unaffected by the addition of glycerin at 0, 2, or 4% of cannulated steer diets (linear,  $P > 0.51$ ). Apparent total tract digestibilities of NDF tended to decrease as glycerin concentrations increased to 2 and 4% (linear,  $P < 0.13$ ). Ruminal pH increased as glycerin concentrations increased (linear,  $P < 0.05$ ), and concentrations of butyrate and valerate decreased (linear,  $P < 0.03$ ). Acetate production also tended to decrease when glycerin increased from 0 to 2 or 4% of the diet (linear,  $P = 0.06$ ). Collectively, these results suggest that glycerin may negatively influence fiber digestion.

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## Preface

This dissertation includes a literature review and three research chapters. The literature review provides an overall summary of crude glycerin fed to beef cattle. Chapter II contains data obtained from research of feedlot heifers evaluating the effects of crude glycerin on feedlot performance and carcass characteristics. Chapter III contains data from cannulated steers evaluating the effects of crude glycerin on ruminal metabolism and diet digestibility. Appendix A contains data obtained from research of feedlot heifers evaluating the effects of extended withdrawal times of zilpaterol hydrochloride on feedlot performance, carcass characteristics, and meat tenderness. All chapters were prepared to follow the guidelines suggested for contributors to the *Journal of Animal Science*.

**CHAPTER 1 - Effects of crude glycerin in livestock production: A  
Review**

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## **Introduction**

Glycerin (glycerol) was accidentally discovered in 1779 by a Swedish chemist named K. W. Scheele by heating a combination of olive oil and lead monoxide (The Soap and Detergent Association, 1990). Glycerin didn't become economically or industrially significant until 1866 with the invention of dynamite. Use of glycerin allowed for the safe transportation of explosives and gained importance in the military for its first economical use. Currently, glycerin has over 1,500 industrial uses, which include the production of synthetic polymers, cosmetics, personal care products, food, plastic and alkyd resins, and pharmaceuticals (ASAIM, 2007). Glycerin is produced as a by-product of soap production from vegetable oils or animal fats, and more recently, in biodiesel production (transesterification). Three processes are available for alkyl ester production: 1) oil conversion to fatty acids followed by acid-catalyzed esterification; 2) base-catalyzed transesterification with methanol; and 3) direct acid-catalyzed esterification with methanol. The most economical process is base-catalyzed transesterification, which is the preferred process utilized for biodiesel production (Van Gerpen, 2005). Base-catalyzed biodiesel production is achieved by mixing fats or oils with a short chain alcohol (methanol) and a catalyst (sodium or potassium hydroxide), where the alcohol and catalyst are usually pre-mixed. Remaining methanol is captured by distillation, and glycerin is recovered through evaporation. Approximately 10% of the weight of oil used for biodiesel production is converted to glycerin or about 0.3 kg per 3.78 liters of biodiesel produced (Thompson, 2006). Glycerin from biodiesel production contains many impurities and requires further refining to be suitable for many manufacturing processes. Alternative uses that don't require additional refining of crude glycerin have been sought since the biodiesel boom.

## **Increases in Biodiesel and Glycerin Production**

Rapid expansion of the biodiesel industry over the past decade has resulted in an increase in availability of crude glycerin. Production of biodiesel in the United States rose from 1.89 million liters in 1990 to approximately 2.65 billion liters in 2008 due to implementation of tax incentives (National Biodiesel Board, 2010). Increased biodiesel production increased availability of crude glycerin to approximately 30 million kg in 2008. In 2007, the refined glycerin market was able to produce about 90.9 billion kg of refined glycerin globally, and the American market produced approximately 181.8 million kg, compared to domestic usage of approximately 159 million kg (American Soybean Association International Marketing, 2007). Increasing availability of crude glycerin drove prices downward, causing unprofitable refiners to close down and creating excesses of glycerin for other uses such as animal feed. In 2006, glycerin that once sold between 9 to 11 cents per kg was available for around 3 cents and lower (Nilles, 2006). The combination of inexpensive glycerin and record corn prices provided an impetus for producers to evaluate glycerin as an alternative feed resource. Tax incentives for biodiesel production expired on January 1<sup>st</sup> 2010, causing plants to cease production, ultimately affecting availability and market value of glycerin. Utilization of crude glycerin in livestock production will be limited to the price of the commodity compared to competing carbohydrate sources.

### **Glycerin Composition**

In its pure form, glycerin is a sweet, odorless, colorless liquid that is very viscous, hygroscopic, and has a high boiling point. Based upon purity and end use, there are three main grades of crude glycerin: 1) Technical grade- not for food or pharmaceutical use but for use in chemicals; 2) United States Pharmacopeia (USP) – suitable for food and pharmaceutical production; 3) Kosher – glycerin from plant sources that can be used for the manufacturing of

Kosher food products, and are generally greater than 99% pure glycerin (American Soybean Association International Marketing, 2007). In the crude form glycerin ranges from light amber to dark brown due to impurities. Crude glycerin is approximately 60 to 85% pure, with the remainder composed of salt, ash, methanol, lipid, and water. Concentrations of impurities are highly variable due to the catalyst used during production, methanol recovery rate, and proportion of remaining lipids. According to Gott (2009), crude glycerin samples from the production of biodiesel contained a mean concentration of 4.79% ash, with a range of 1.28 to 8.98%. Likewise, Thompson et al. (2006) noted ash concentrations ranging from 0.65 to 5.5%, sodium ranging from 1.06 to 1.40%, lipids from 1.1 to 60.1%, carbohydrates ranging from 26.9 to 83.8%, and protein levels ranging from 0.05 to 0.44% in crude glycerin samples obtained from multiple feedstocks. In the aforementioned study, the variation in the chemical composition of crude glycerin is less when the feedstocks used are neat oils compared to utilizing waste vegetable oils. Kerr et al. (2007) reported methanol contents of two crude glycerin samples obtained from one production plant in May and August 2006 contained 0.03 and 0.32% methanol, respectively. According to Gordon (2009), FDA indicated methanol can range from 5 to 20,000 mg/kg and sodium sulfate (salt) reached as much as 16,000 mg/kg in crude glycerin intended for animal feed. Variation in methanol content of crude glycerin can depend upon the amount of methanol used in production. Food and Drug administration has established an upper limit of methanol content in crude glycerin at 150 mg/kg, indicating it must meet U.S. Pharmacopeia (U.S.P.) standards for glycerin. The Federal Republic of Germany has stated that methanol levels up to 5,000 mg/kg in glycerin are safe for animal production (Sellers, 2008). The previous author states the crude glycerin is a GRAS animal food ingredient according to Title 21, Code of Federal Regulations, Section 582.1320. Concentrations of methanol in crude

glycerin is acceptable at levels up to 1,000 mg/kg in Canada, 5,000 mg/kg in European Union, and 1% of the diet or 10,000 mg/kg in the state of Texas, U.S.A (Gordon, 2009). Significant variation in the chemical composition of crude glycerin can make diet formulation in livestock production challenging. At this point, it is unclear how the differences in composition may affect animal performance and at what level methanol concentrations become harmful.

### **Ruminal Fermentation of Glycerin**

Glycerin can negatively affect cellulolytic activity in the rumen, ultimately decreasing fiber digestion. Reductions in cellulose degradation by cellulolytic bacteria and cellulolytic fungi were observed *in vitro* when media contained 0.5% and 5% glycerin, respectively (Roger et al., 1992). Supporting this, glycerin reduced IVDMD of oat hay and carboxymethyl-cellulose, which is a soluble substrate less complex than forage (Paggi et al., 2004). Inhibitory effects of cellulolytic activity were similar to the glycerin concentrates in the media of Roger et al. (1992) trial where they inhibited fungal activity. Likewise, Parsons and Drouillard (2010) observed a tendency (linear,  $P = 0.12$ ) towards a reduction in apparent total tract digestion of NDF when cannulated steers were fed crude glycerin in the diet at 2 and 4% (DM basis). In contrast, Hess et al. (2008) reported no change in fiber digestion of warm-season grasses when glycerin was added at 15% of the media *in vitro*, but noted decreases in fiber digestion of cool-season grasses. According to Krehbiel (2008), differences in digestibility of fiber could be attributed to the ability of microorganisms to adapt to glycerin over time, as absorption of glycerin increases over time. *In vitro* studies may not accurately reflect *in vivo* conditions since microorganisms may not have sufficient time to adapt. Cannulated steers used by Parsons and Drouillard (2010) were adapted to glycerin concentrations for 10 d prior to sampling, which may not have been sufficiently long for manifestation of changes in glycerin absorption or microbial adaptation.



Concerns of fiber digestibility are limited in feedlot cattle fed finishing diets because fiber concentrations normally are low, but decreasing fiber digestion in starter diets, step-up diets, or diets containing large portions of distiller's grains could potentially impact animal performance negatively.

Protein metabolism has been compromised when glycerin has been added to portions of the medium or when ruminally dosed. Increasing media concentrations of glycerin from 50 mM to 300 mM *in vitro* decreased proteolytic activity within ruminal fluid by approximately 20% at all glycerin concentrations (Paggie et. al., 1999). Likewise, decreases in bacterial protein synthesis and branched chain VFA concentrations were noted when Kiljora et al. (1998) ruminally dosed 200 g of glycerin twice daily for 6 d. At the present time there is no clear understanding of differences observed in digestibility of diet components except that the glycerin utilized in each trial had different chemical compositions, which could play a significant role in ruminal fermentation. Better understanding of the glycerin composition utilized in each trial could potentially reveal which component of crude glycerin alters ruminal fermentation.

### **Ruminal Metabolism of Glycerin**

Currently, it's unclear how synthesis of volatile fatty acids (VFA) in the rumen maybe influenced when glycerin is rapidly metabolized. Garton et al., (1961) reported that glycerol was fermented to VFAs *in vitro*, but could account for only half of the glycerin that was metabolized, with propionic acid constituting the majority of VFA produced. Supporting this, Johns (1953) found increases in propionic acid *in vitro* and *in vivo* by sheep rumen contents when glycerol was added. Incubating glycerol with rumen contents from cattle resulted in increases in acetic and propionic acids as the main end products of glycerol metabolism (Wright, 1969). Other researchers noted increases in propionic and butyric acid at the expense of acetic acid production

when glycerin was included (Czerkawski and Breckenridge, 1972; Kijora et al., 1998; and Rémond et al., 1993). Feeding crude glycerin at 0, 2, or 4% (DM basis) in finishing diets to cannulated steers decreased (linear,  $P \leq 0.06$ ) acetate, butyrate, and valerate concentrations as glycerin level increased, but propionate concentrations were unchanged due to dietary treatment (Parsons and Drouillard, 2010). Supporting this, Trabue et al. (2007) found that acetate production somewhat decreased and propionate concentrations were unaffected when glycerol was mixed with rumen fluid from a dairy cow consuming a diet consisting of approximately 50% concentrate and 50% forage. No changes in total VFA production were observed when glycerin was added to high concentrate diets fed to cattle (Parsons and Drouillard, 2010; Mach et al., 2009). Lactic and succinic acids are metabolites that also can be derived from glycerol fermentation (Hobson and Mann, 1961; Garton, 1963; Stewart and Bryant, 1988). As well, Jarvis et al. (1997) reported that equimolar proportions of formate and ethanol were formed from glycerin by *Klebsiella planticola* when rumen contents of red deer were utilized. Glycerin can be metabolized into a wide range of end products, differences may be due to diet type and the rumen microflora present.

### **Glycerin in Livestock and Poultry Diets**

Utilization of crude glycerin can help with texturing effects of livestock diets by agglomerating small feed particles, controlling dust, and reducing fines. Glycerin has been observed to decrease energy costs associated with pelleting corn-based swine diets when added at up to 15% of the mash (Groesbeck et al., 2008). The same author reported that optimal pellet durability indices (PDI) were achieved with approximately 9% glycerin. Several studies have evaluated the effects of glycerin as an energy source for poultry (Simon et al., 1996; Cerrate et al., 2006), swine (Kijora et al., 1995; Lammers et al., 2008), sheep (Gunn et al., 2010), and cattle

(DeFrain et al., 2008; Pyatt et al., 2007). Crude glycerin feeding rates to livestock have ranged from 0 to 20% of the dietary dry matter in published literature.

Utilizing crude glycerin in livestock diets has led to inconsistent results. Parsons et al. (2009) reported no differences in DMI when the diet contained 2% glycerin, but reductions (linear,  $P < 0.001$ ; quadratic,  $P = 0.014$ ) in DMI occurred when glycerin levels increased to 4, 8, 12, and 16% of a steam-flaked corn finishing diet. Likewise, DMI decreased 10.1% when crude glycerin was included at 10% of dry-rolled corn diets and fed to steers (Pyatt et al., 2007). Elam et al. (2008) observed linear reductions in DMI when glycerin levels were increased to 7.5 and 15% of the diet. Parturient dairy cows fed 5% glycerin had greater DMI, but with 3.3% glycerin DMI decreased after calving (Ogborn, 2006). In contrast, Mach et al. (2009) reported no differences in DMI of Holstein bulls fed barley-based diets consisting of up to 12% glycerin. Furthermore, studies utilizing dairy cattle revealed no impact on feed intake when glycerin was included at up to 10% of dietary dry matter in high-forage diets (Schröder and Südekum, 1999; DeFrain et al., 2004; Chung et al., 2007). In finishing lambs, feeding crude glycerin at up to 20% of the diet had no effect on DMI (Gunn et al., 2010). Feeding increased levels of crude glycerin might change diet digestibilities and alter rumen microflora populations. Groesbeck et al. (2008) noted increases in ADFI for pigs fed pelleted diets containing crude glycerin when compared to pelleted diets containing soybean oil ( $P = 0.08$ ). Other research noted no differences in ADFI when glycerin was fed in diets containing corn, barley, or wheat with soybean meal (Mourot et al., 1994; Kijora et al., 1995; Lammers et al., 2008). In poultry, feeding glycerin at 5% of the diet had no effect on feed intake, but increasing glycerin to 10% of the diet decreased feed intake, presumably due to poor flowability of the mash diet (Simon et al., 1996; Cerrate et al., 2006).

Average daily gains increased by 12.6, 8.4, and 5.0% when glycerin replaced 2, 4, and 8% of steam-flaked corn, respectively (linear  $P = 0.013$ ; quadratic,  $P = 0.010$ ), but decreased ADG in finishing heifers by 1.7 and 13.4% when glycerin was 12 and 16% of the diet, respectively (Parsons et al., 2009). Pyatt et al. (2007) reported an increase of 11.4% in ADG when glycerin replaced 10% of the dry-rolled corn, but only improved ADG by 2.5% when 10% glycerin was fed in dry-rolled corn diets that also contained 30% distiller's grains with solubles and 15% soy hulls. Similarly, replacing barley with 5 and 10% crude glycerin in finishing pig diets increased ADG by 5.3 and 12%, respectively, but increasing glycerin to 20 and 30% of the diet decreased ADG by 3.7 and 18.2%, respectively (Kijora et al., 1995). Groesbeck et al. (2008) reported linear increases in ADG in nursery pigs when glycerin concentrations increased from 0 to 3 or 6% of the diet. Donkin et al. (2007) reported increases in BW gains in lactating dairy cows when glycerin replaced 10 or 15% of the corn in diets consisting of corn silage, legume forage, and corn grain. Feeding glycerin up to 12% of the diet had no effect on ADG of Holstein bulls (Mach et al., 2009). Likewise, Gunn et al. (2010) reported no differences in ADG in finishing lambs when glycerin was included up to 20% of the diet. In finishing pigs, Duttlinger et al. (2008) found no differences in ADG when glycerin was fed at either 2.5 or 5% of the ground corn-based diet. Body weights of broilers were similar when glycerin was included at up to 5% of the diet, but significant decreases in body weights occurred when glycerin was fed at 10% for 42 d (Cerrate et al., 2006). Low to moderate levels of glycerin can potentially increase growth. Decreases in ADG may be attributed to the decreases in ADFI that some researchers have observed.

Feed efficiency has been observed to improve with moderate levels of crude glycerin. Pyatt et al. (2007) reported a 21.9% improvement in efficiency when 10% of the dry-rolled corn

was replaced with glycerin, and a 16.4% improvement when 10% glycerin replaced dry-rolled corn and fed in combination with 30% dried distillers grains with solubles and 15% soyhulls. Likewise, Parsons et al. (2009) noted improvements in efficiency by 10.8, 10.0, 7.2, and 3.1% when glycerin replaced steam-flaked corn at levels of 2, 4, 8, and 12%, respectively, but decreased efficiency by 2.8% when fed at 16% of the finishing diet. Mach et al. (2009) reported no effect on G:F when glycerin was fed at up to 12% of the diet to Holstein bulls. In broilers, efficiency improved by 1.0 and 0.9% when glycerin was added at 2.5 and 5% (Cerrate et al., 2006). Kijora et al. (1995) noted decreases in growth efficiency in swine when glycerin was included at rates greater than 10% of the diet. Other swine studies reported a tendency to improve G:F (Duttlinger et al., 2009) or no improvement when glycerin comprised 5 or 10% of diets (Lammers et al., 2008). Gunn et al. (2010) reported improvements in efficiency the first 14 d on trial, but no differences in feed efficiency were detected when lambs were fed 0, 5, 10, 15, or 20% crude glycerin over the entire period of 84 d. Feeding low levels of glycerin in livestock production can occur without negatively impacting feed efficiencies, and in some cases can yield substantial improvements.

Glycerin is thought to have gluconeogenic properties (Bergman et al., 1968) and could potentially improve carcass quality grades. Current research has suggested that the glucogenic properties do not occur with glycerin utilization or the intake of glycerin in livestock diets are insufficient to elicit changes. Parsons et al. (2009) noted linear ( $P = 0.022$ ) decreases in marbling scores when glycerin was included in the diets compared to control cattle, and reductions in marbling scores resulted in a tendency (linear,  $P = 0.084$ ) to decrease the percentage of carcasses that graded USDA Choice, while increasing the percentage of USDA Select carcasses. Likewise, Elam et al. (2008) observed an increase in the percentage of cattle

grading USDA Select due to feeding glycerin. Mach et al. (2009) noted that bulls fed diets containing 8% glycerin had numerically the greatest intramuscular fat content when compared to control bulls. Research is limited on the effects of glycerin in respect to marbling scores and USDA carcass grades.

Hot carcass weights of cattle were increased by 8.1, 5.1, and 3.2 kg when glycerin was fed at 2, 4, and 8%, respectively (linear,  $P = 0.009$ ; quadratic,  $P = 0.013$ ), but decreased 1.2 and 9.1 kg when glycerin replaced 12 and 16% of steam-flaked corn, respectively (Parsons et al., 2009). Mach et al. (2009) reported no differences in HCW of Holstein bulls that were fed up to 12% crude glycerin. Likewise, Gunn et al. (2010) showed no differences in HCW of wethers fed finishing diets containing up to 20% glycerin. In swine, Lammers et al. (2008) indicated that glycerin concentrations had no effect on HCW. At low concentrations, glycerin has been observed to improve or not affect HCW when used in animal production.

Longissimus muscle area of cattle increased when glycerin was fed at 2% of the diet, but decreased as glycerin concentrations increased (Parsons et al. 2009). Cerrate et al. (2006) noted increases in breast meat yield of 0.64 and 0.80% in broilers fed 2.5 and 5% glycerin, respectively. No differences in LM area was noted in Holstein bulls fed up to 12% glycerin (Mach et al., 2009), lambs fed up to 20% glycerin (Gunn et al., 2010), or pigs fed up to 10% glycerin (Lammers et al., 2008). Changes in muscle size have not been well defined in livestock production due to limited research.

## **Summary**

Feeding glycerin in livestock production has yielded variable results. Some studies indicate that glycerin can be fed at relatively higher concentrations with positive results, while other researchers have noted negative results when glycerin concentrations exceeded 10% of diet

dry matter. Differences in biodiesel feedstocks, chemical compositions of crude glycerin, and the energy value of glycerin may contribute to the variability of results. A better understanding of glycerin metabolism, changes in rumen microflora, and potential dietary interaction could help explain differences in performance and shed light on proper feeding levels for the future. Short term, glycerin prices will continue to rise in 2010 due to the decrease in biodiesel production, which most likely will result in unfavorable pricing for livestock diets. Increases in the biodiesel industry will likely continue with the renewal of the biodiesel tax, which again will create an excess supply of glycerin. Feeding glycerin levels greater than 10% of the diet may have a deleterious effect on livestock performance and result in unfavorable carcass characteristics.

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**CHAPTER 2 - Performance and carcass traits of finishing heifers  
fed crude glycerin**

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## ABSTRACT

Crossbred heifers ( $n = 373$ ;  $421.6 \text{ kg} \pm 28.9$ ) were fed finishing diets containing 0, 2, 4, 8, 12, or 16% crude glycerin (DM basis). Diets consisted of steam-flaked corn with 6% alfalfa hay and 1.2% urea and provided 300 mg monensin, 90 mg tylosin, and 0.5 mg melengestrol acetate per animal daily. Cattle were stratified by body weight and allocated randomly, within strata, to concrete-surfaced feedlot pens each containing 6 to 7 heifers with 9 pens per dietary treatment. Cattle were transitioned from the control diet to diets containing increasing proportions of glycerin over a period of 10 d. Cattle had ad libitum access to feed, and diets were delivered once daily throughout the 85-d trial period. As the level of glycerin increased DMI decreased linearly ( $P < 0.001$ ). Heifers fed 0, 2, 4, 8, 12, and 16% glycerin had ADG of 1.19, 1.34, 1.29, 1.25, 1.17, and 1.03 kg/d, respectively (Lin,  $P = 0.013$  and Quad,  $P = 0.010$ ). Feeding glycerin had a quadratic effect on efficiency of gain, and efficiency of gain was optimal when glycerin was fed at 2% of the diet (Quad,  $P = 0.046$ ). Glycerin increased final BW by 12.7, 8.1, and 5.3 kg when fed at 2, 4, and 8% of the diet, respectively but reduced final BW 1.9 and 14.3 kg when included at 12 and 16% of the diet (Lin,  $P = 0.009$ ; Quad,  $P = 0.006$ ). Similarly, HCW increased by 8.1, 5.1, and 3.3 kg when glycerin was fed at 2, 4, and 8% of the diet, respectively but were 1.2 and 9.1 kg less than controls when glycerin was fed at 12 and 16%, respectively (Lin,  $P = 0.009$ , and Quad,  $P = 0.006$ ). Longissimus muscle area decreased linearly as glycerin levels increased ( $P < 0.013$ ). Feeding glycerin resulted in linear decreases in subcutaneous fat over the 12th rib and marbling scores ( $P = 0.045$ ). Glycerin tended to decrease the percentage of cattle grading USDA Choice ( $P = 0.084$ ) and increase the percentage of cattle grading USDA Select. Adding glycerin to cattle finishing diets improved weight gain and efficiency, particularly when added at levels of 8% or less of DM basis.

Keywords: Glycerin, Heifers, Steam-Flaked Corn

## INTRODUCTION

Due to rising corn costs, alternative feed sources, such as glycerin, have become a major focus for the livestock industry. Rapid expansion of the biodiesel industry created affordable supplies of crude glycerin. Catalyzed reactions between alcohol and triglycerides in vegetable oils and animal fats yields biodiesel and the co-product, crude glycerin (Van Gerpen, 2005). Approximately 10% of the weight of oil or fat used to produce biodiesel becomes glycerin (Darasi et al., 2005), and the U.S. biodiesel industry anticipates glycerin output to be 635 million kg between 2006 and 2015.

Several studies evaluated use of glycerin in diets for poultry (Simon et al., 1996 and Cerrate et al., 2006), swine (Kijora et al., 1995; Lammers et al., 2007a, 2007b), and cattle (Fisher et al., 1973 and DeFrain et al., 2004). In non ruminants limited work has been conducted to understand metabolism of glycerin. On ingestion, glycerol is converted to glucose via phosphorylation to glycerol-3-phosphate (G3P), which is catalyzed by glycerol kinase and enters gluconeogenesis in the liver (Mourot et al., 1994). Trabue et al. (2007) suggest that ruminal metabolism of glycerol is approximately 80% after 24 h, resulting in a decreased acetate:propionate ( $P < 0.05$ ) ratio (Schröder and Südekum, 2007). Our lab estimates that glycerin is almost entirely converted to propionate this would support a reduction in acetate:propionate ratio (Unpublished data). Previous research suggests glycerin is an effective feed source in multiple species, but limited data shows the effect of glycerin on beef cattle performance and carcass characteristics.

Our objective was to evaluate effects of glycerin on feedlot performance and carcass characteristics while establishing an optimal feeding level in finishing heifers fed diets containing steam-flaked corn.

## MATERIALS AND METHODS

Procedures for this experiment were approved by the Kansas State University Institutional Animal Care and Use Committee. Crossbred heifers ( $n = 373$ ;  $421.6 \text{ kg} \pm 28.9$  initial BW) were used in a randomized complete block design to determine the effects of crude glycerin in finishing diets. On arrival, all cattle were offered ad libitum access to alfalfa hay and water before processing. Within 24 h of arrival, cattle received injections of Bovishield 4 and Ultrabac 7 vaccines (Pfizer Animal Health; Exton, PA) and were treated with Phoenectin parasiticide (IVX Animal Health; St. Joseph, MO). Cattle were implanted with Revalor-200 (Intervet; Millsboro, DE), and gradually adapted to a 94% concentrate diet with 6% alfalfa hay (Table 1). Cattle were blocked by initial weight and randomly assigned, within block, to each of the six treatments. Three weight blocks were used, with 6 to 7 animals per pen and 9 pens per treatment for a total of 54 pens. Cattle were housed on concrete-surfaced pens ( $36.5 \text{ m}^2$ ) with roofs covering half the pen and the entire feed bunk. All diets contained 30 mg of monensin/kg, 9 mg of tylosin/kg (Elanco Animal Health, Greenfield, IN), and 0.5 mg melengesterol acetate per (Pfizer Animal Health; Exton, PA) heifer daily. Dietary treatments consisted of 0, 2, 4, 8, 12, or 16% soy-based crude glycerin (DM basis) in steam-flaked corn diets. Cattle were transitioned from the control diet to diets containing increasing proportions of glycerin over a period of 10 d. Cattle were fed once daily (afternoon) ad libitum. Heifers were fed for 85 d and then transported to a commercial abattoir where carcass data were collected. Hot carcass weights and liver scores were obtained at the time of harvest. Longissimus muscle area; 12th rib fat thickness; marbling score; kidney, pelvic, and heart fat; incidences of dark cutters; and USDA quality and yield grades were collected following a 24-h chill. Carcass adjusted final body weight (CBW) was calculated by dividing hot carcass weight by a common dressing percentage of 63.5%.



### *Statistical Analysis*

Data were analyzed as a randomized complete block design using the Mixed model procedure of SAS (SAS Inst. INC., Cary, NC). Pen was the experimental unit, and model effects included block and treatment. Orthogonal contrasts were used to determine linear, cubic, and quadratic effects of glycerin and 0 versus glycerin. Treatment means were computed with the LSMEANS option.

## **RESULTS**

### *Feedlot Performance*

Average daily gains increased by 12.6, 8.4, and 5.0% for cattle fed 2, 4, and 8% glycerin, respectively, but at 12 and 16% glycerin, ADG were reduced by 1.7 and 13.4%, respectively (Table 2, Lin,  $P = 0.013$  and Quad,  $P = 0.010$ ). First reports of glycerin fed in finishing steers diets resulted in improving ADG by 11.4% when glycerin replaced 10% of the dry rolled corn, but only improved ADG by 2.5% when glycerin replaced 10% of the dry rolled corn in diets that also contained 30% DDGS and 15% soy hulls (Pyatt et al. (2007). Similarly, feeding glycerin to finishing pigs at 5 and 10% of the diet increased ADG by 5.3 and 12%, respectively, but replacing barley with 20 and 30% glycerin decreased ADG by 3.7 and 18.2%, respectively (Kijora et al., 1995). In lactating dairy cows fed diets consisting of corn silage, legume forage, and corn grain, replacing corn with 10 and 15% glycerin increased weight gains (Donkin et al., 2007). Low to moderate levels of glycerin, particularly less than 8%, in feedlot diets effectively increases daily weight gains.

No changes in DMI occurred when glycerin was fed at either 0% or 2% of the diet (8.84 versus 8.88 kg), but increasing glycerin to 4, 8, 12, and 16% reduced DMI to 8.66, 8.61, 8.40, and 7.80 kg, respectively (Lin,  $P < 0.001$  and Quad,  $P = 0.014$ ). Similarly, Pyatt et al. (2007)

reported a 10.1% reduction in DMI when glycerin was added at 10% to a dry rolled corn diet fed to steers. Kijora et al. (1995) reported DMI of finishing hogs increased by 9.6 and 12.6% when glycerin replaced 5 and 10% barley. Schröder and Südekum (2007) reported a 0.7 kg/d reduction in starch intake in ruminally cannulated steers fed 15% glycerol. Changes in DMI and replacing rapidly fermentable starch sources with glycerin could explain the reductions in total ingested starch. Trabue et al. (2007) reported that increases in lactate accumulation might slow glycerol fermentation in the rumen, altering intake. In addition, Roger et al. (1992) reported that adding glycerin at 5% of the in vitro media greatly inhibited growth and cellulolytic activity of rumen bacteria and fungus. Small inclusions of glycerin could be beneficial to livestock growth, but levels greater than 5% might create an unhealthy rumen, resulting in reduced DMI.

Feed efficiency improved by 10.8, 10.0, 7.2, and 3.1% when glycerin was included at 2, 4, 8, and 12% of the diet, respectively, but adding glycerin at 16% reduced efficiency by 2.8% (Quad,  $P = 0.046$ ). Pyatt et al. (2007) reported a 21.9% improvement in efficiency when glycerin replaced 10% of the dry-rolled corn in the diet and a 16.4% improvement when glycerin replaced 10% of the dry-rolled corn in diets also contain 30% distillers grains. In broilers, glycerin improved feed efficiency by 1.3% compared with controls when fed at 5% but reduced feed conversion by 3.1% when added at 10% of the diet and fed for 42 d (Cerrate et al., 2006). Researchers who conducted that study commented that the decrease in efficiency and growth at the 10% level was the result of diets with poor flowability that lodged in feeders. Another compared levels of 0, 2.5, and 5% glycerin in male broiler diets fed for 42 d; efficiency improved 1.1 and 0.9% over controls when glycerin was added at 2.5 and 5%, respectively.

Increasing dietary levels of crude glycerin cause linear reductions in shrunk final BW (Lin,  $P = 0.001$ ) increased at 2 and 8% glycerin but decreases in final BW were observed at 4,

12, and 16% glycerin (Quad,  $P = 0.017$ ). Cerrate et al. (2006) reported no differences in BW when glycerin was fed at 5% to male broilers, but a significant reduction in body weight occurred when glycerin was fed at 10% of the diet. Their second experiment resulted in final BW increasing by 3.59 and 3.48% over controls when glycerin was fed at 2.5 and 5%, respectively (Cerrate et al., 2006). Glycerin can have positive effects on ADG, efficiency, and final BW when included at less than 10% of livestock and poultry diets.

### ***Carcass Characteristics***

Carcass adjusted final BW increased 12.7, 8.1, and 5.3 kg when glycerin was fed at 2, 4, and 8 % glycerin, respectively but glycerin decreased body weight by 1.9 and 14.3 kg when fed at 12 and 16%, respectively. Likewise, increases in HCW by 8.1, 5.1, and 3.2 kg were observed when glycerin was fed at 2, 4, and 8%, respectively but decreased HCW by 1.2 and 9.1 kg when fed at 12 and 16%, respectively (Table 3, Lin,  $P = 0.009$  and Quad,  $P = 0.006$ ). Including glycerin at up to 8% of the diet could effectively increase HCW in finishing cattle. Longissimus muscle area significantly increased when glycerin was fed at 2% of the diet, but a linear ( $P = 0.013$ ) reduction in LM area occurred with increasing amounts of glycerin. Similarly, chickens fed glycerin at 2.5 and 5% levels showed significant increases of 0.64 and 0.80% breast meat yield, respectively (Cerrate et al., 2006).

Heifers fed glycerin at 2 and 16% were leaner and had lower numerical USDA yield grades, but feeding glycerin at intermediate levels had no effect on yield grades (Cubic,  $P = 0.050$ ). Glycerin caused linear ( $P = 0.022$ ) reductions in marbling scores compared with control heifers. Previous research suggests that increasing the glucogenic substrates (e.g. glycerin) fed to cattle results in increased marbling scores. However, glycerin showed no positive marbling benefits when fed at various concentrations to feedlot heifers. Glycerin tended (Lin,  $P = 0.084$ )

to decrease the percentage of cattle grading USDA Choice while simultaneously increasing the percentage of cattle grading USDA Select. Reductions in USDA quality grades might be associated with the decline in subcutaneous fat due to fed diets glycerin ( $P = 0.096$ ). Glycerin fed cattle were leaner, the most notable reductions in subcutaneous fat occurred when glycerin was fed at 2 and 16% (Lin,  $P = 0.045$  and Cubic  $P = 0.009$ ). The reductions in subcutaneous fat for the two aforementioned treatments might explain the lower numerical yield grades, but at other dietary levels glycerin elicited no effect on yield grade even with leaner carcasses. Since glycerin reduced subcutaneous fat it is conceivable that glycerin may alter fat deposition, which might explain the observed reductions in marbling scores. Since a majority of glycerin is converted to propionate we speculated that improvements in quality grades would be observed, but this proved untrue because glycerin fed cattle were leaner and had lower marbling scores. No treatment differences occurred for KPH percentages and liver abscesses. Our data indicates that in finishing diets glycerin levels up to 8% are optimal and greater levels can have deleterious effects. Supporting this, Schröder and Südekum (2007) suggest that glycerin concentrations greater than 10% of diet DM will affect feed intake, water intake, and digestibility of the diet and nutrients. Reductions in feed intake might explain the decreases in live performance and changes in carcass characteristics observed in our study when glycerin was fed at 12 and 16%. Feeding less than 8% crude glycerin in steam-flaked corn diets can improve gain and efficiency of finishing heifers, with maximum benefits observed at the 2% level. Understanding the effects of glycerin on meat characteristics, VFA, ruminal changes, and digestibility in ruminants will be necessary to determine glycerin relative feed value. As well, this will help answer questions pertaining to differences in performance and carcass characteristics as inclusion rates increase.

**Table 2-1 Experimental diets (DM basis) and calculated dietary nutrients for cross-bred heifers fed diets containing 0, 2, 4, 8, 12, and 16% crude glycerin on a dry basis**

Ingredient, %	Crude glycerin, %					
	0	2	4	8	12	16
Steam-flaked corn	82.6	80.2	77.8	73.0	68.2	63.4
Corn steep liquor	5.7	5.7	5.7	5.7	5.7	5.7
Alfalfa hay	5.9	5.9	5.9	5.9	5.9	5.9
Crude soy-based glycerin <sup>1</sup>	0.0	2.0	4.0	8.0	12.0	16.0
Soybean meal	0.37	0.80	1.20	2.03	2.87	3.69
Limestone	1.45	1.46	1.44	1.42	1.41	1.40
Urea	1.15	1.14	1.14	1.13	1.13	1.11
Salt	0.28	0.29	0.28	0.28	0.28	0.27
Mineral premix <sup>2</sup>	0.35	0.34	0.34	0.34	0.31	0.33
Feed additive premix <sup>3</sup>	2.2	2.2	2.2	2.2	2.2	2.2
Nutrients						
DM	81.0	81.2	81.3	81.5	81.7	81.9
CP	14.9	14.9	14.8	14.7	14.6	14.5
Ca	0.66	0.67	0.67	0.67	0.67	0.67
P	0.32	0.32	0.31	0.30	0.28	0.27

<sup>1</sup>Methanol content of glycerin <0.01%

<sup>2</sup>Formulated to contain 0.1 mg/kg Co; 10 mg/kg Cu; 0.6 mg/kg I; 60 mg/kg Mn; 0.25 mg/kg Se; 60 mg/kg Zn; 1.0% K; 2,640 IU/kg vitamin A; and 220 IU/kg vitamin E.

<sup>3</sup>Feed additive premix was formulated to provide 300 mg monensin, 90 mg tylosin, and 0.5 mg melengestrol acetate per heifer daily in a ground corn carrier. Additionally, ractopamine-HCl was included at 200 mg/d the final 42 d prior to harvest.

**Table 2-2 Feedlot performance of heifers fed 0, 2, 4, 8, 12, 16% crude glycerin for final 85 days on feed**

Item	Crude Glycerin, %						SEM	Contrasts P-values		
	0	2	4	8	12	16		Linear	Quadratic	0 vs Glycerin <sup>6</sup>
Number of heifers	62	62	61	63	63	62	-	-	-	-
Days on feed	85	85	85	85	85	85	-	-	-	-
Initial weight, kg	421.7	421.6	421.4	421.7	421.8	421.6	5.74	0.990	0.992	0.991
Shrunk final weight <sup>4</sup> , kg	528.0	531.2	526.2	530.3	522.1	508.0	4.303	0.001	0.017	0.342
Final weight <sup>5</sup> , kg	523.0	535.7	531.1	528.3	521.1	508.71	7.31	0.009	0.006	0.732
DMI, kg	8.84	8.88	8.66	8.61	8.40	7.80	0.13	0.001	0.014	0.015
ADG, kg	1.19	1.34	1.29	1.25	1.17	1.03	0.09	0.013	0.010	0.741
G:F	0.1362	0.1516	0.1496	0.1458	0.1397	0.1324	0.007	0.319	0.046	0.320

<sup>4</sup>Calculated by multiplying final body weight by 96 percent

<sup>5</sup>Calculated by dividing HCW by a common dressing percentage of 63.5.

<sup>6</sup>Compares the effects of 0% glycerin to combination glycerin treatment

**Table 2-3 Carcass characteristics of heifers fed 0, 2, 4, 8, 12, 16% crude glycerin**

Item	Crude Glycerin,%						SEM	Contrasts P-values			
	0	2	4	8	12	16		Linear	Quadratic	Cubi	0 vs Glycerin
Hot carcass weight, kg	332.1	340.2	337.3	335.4	330.9	323.0	4.6	0.009	0.006	0.54	0.732
Dresses yield, %	63.0	64.1	64.2	63.3	63.4	63.6	0.005	0.924	0.328	0.08	0.161
LM area, cm <sup>2</sup>	83.1	86.3	84.0	82.7	81.7	81.4	1.5	0.013	0.217	0.05	0.903
USDA yield grade											
Yield grade 1,%	11.4	16.1	13.2	11.1	12.7	15.9	4.03	0.767	0.787	0.31	0.584
Yield grade 2,%	32.0	32.8	31.2	33.3	28.6	40.5	6.5	0.563	0.476	0.47	0.859
Yield grade 3,%	51.9	51.1	47.6	44.5	50.8	42.1	6.6	0.343	0.994	0.70	0.523
Yield grade 4,%	4.8	0.0	6.4	11.1	6.4	1.6	2.6	0.718	0.070	0.02	0.912
Yield grade 5,%	0.0	0.0	1.6	0.0	0.0	0.0	0.7	0.771	0.291	0.46	0.657
Average yield grade	2.5	2.4	2.5	2.6	2.5	2.3	0.09	0.589	0.160	0.05	0.636
USDA quality grade											
Prime, %	3.2	0.0	1.6	0.0	0.0	1.6	1.3	0.377	0.142	0.92	0.076
Choice, %	53.7	50.3	57.4	42.9	52.4	37.3	6.1	0.084	0.389	0.64	0.402
Select, %	43.1	46.5	37.8	53.9	46.0	57.9	5.6	0.064	0.378	0.86	0.386
Standard, %	0.0	3.2	3.2	3.2	1.6	3.2	1.7	0.771	0.291	0.46	0.657
Marbling <sup>7</sup>	435	405	416	398	410	397	9.7	0.022	0.343	0.23	0.008
KPH, %	2.24	2.21	2.19	2.24	2.20	2.19	0.04	0.422	0.889	0.42	0.341
S.C. fat, cm	1.21	1.10	1.18	1.18	1.18	1.02	0.06	0.045	0.125	0.00	0.096
Liver abscess, %	11.11	6.61	17.72	9.52	4.76	17.72	4.10	0.577	0.530	0.15	0.972

<sup>7</sup>Marbling scores were obtained by USDA graders at a commercial abattoir; Slight=300-399, Small=400-499, Modest=500-599.

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**CHAPTER 3 - Effects of crude glycerin on ruminal metabolism and  
diet digestibility in flaked-corn finishing diets**

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## ABSTRACT

The objectives of this study were to determine the effects of crude glycerin on apparent total tract digestibility, and to measure diurnal changes in ruminal pH and concentrations of ammonia and VFA. Crossbred steers ( $n=9$ ;  $624 \pm 80$  kg) fitted with ruminal cannulae were used in a replicated, randomized block experiment with 3 treatments and 3 periods. Treatments consisted of steam-flaked corn diets containing 0, 2, or 4% crude glycerin (DM basis). Steers were allowed *ad libitum* access to finishing diets fed once daily. Diets contained 6% alfalfa hay, and provided 14% crude protein, 3.5% protein equivalent as non-protein nitrogen, 300 mg/d monensin, 90 mg/d tylosin, 2,200 IU/kg vitamin A, 0.3 % salt, 0.7% calcium, and 0.7% potassium. Periods consisted of a 10-d acclimation phase followed by a 3-d collection phase. Chromic oxide (10 g) was used as an indigestible marker to estimate total fecal output, and was dosed intraruminally prior to feeding beginning 7 d prior to the sampling phase. Dry matter intake was similar among treatments ( $P > 0.98$ ). Fecal outputs were 1.21, 1.27, and 1.28 kg/d when glycerin was fed at 0, 2, and 4%, respectively ( $P > 0.74$ ). Apparent total tract digestibilities of DM, OM, starch, CP, and crude fat were similar for cattle fed the different dietary treatments ( $P > 0.51$ ). Apparent total tract digestibilities of NDF were 49.9, 45.8, and 43.4 for cattle fed 0, 2, and 4% glycerin, respectively (Lin,  $P < 0.13$ ). No treatment by time interactions were observed for ruminal parameters ( $P > 0.27$ ). Feeding glycerin linearly increased mean ruminal pH from 5.61 in control steers to 5.67 and 5.73 when glycerin was added at 2 and 4 %, respectively ( $P < 0.05$ ). Concentrations of butyrate and valerate decreased as crude glycerin increased in the diet (Lin,  $P < 0.03$ ). Acetate concentrations decreased with increasing glycerin concentrations (Lin,  $P = 0.06$ ). When fed at low levels in finishing diets, glycerin appears to alter digestion of fiber, but has little impact on other components of the diet.

**Keywords:** Glycerin, Digestibility, Steam-Flaked Corn

## INTRODUCTION

Expansion of the biodiesel industry has increased supplies of crude glycerin that may have direct application in livestock feeding. Catalyzed reactions between methanol and triglycerides from vegetable oils, such as soybean oil, yield biodiesel and the co-product, crude glycerin (Van Gerpen, 2005). Approximately 10% of the weight of soybean oil used to produce biodiesel becomes glycerin. Recent increases in feed costs have inspired livestock producers to seek cost-effective alternatives to traditional feed ingredients. Several studies have evaluated the use of glycerin in poultry (Cerrate et al., 2006), swine (Kijora et al., 1995), dairy cattle (DeFrain et al., 2004), and beef cattle (Parsons et al., 2009). Limited work has been conducted to understand metabolism of glycerin in ruminant livestock. Upon ingestion, glycerol is converted to glucose via phosphorylation to glycerol-3-phosphate (G3P), which is catalyzed by glycerol kinase and enters gluconeogenesis in the liver (Mourot et al., 1994). Trabue et al. (2007) reported that approximately 80% of glycerol is metabolized in the rumen within 24 h, and Schröder and Südekum (2007) observed lower acetate:propionate ( $P < 0.05$ ) ratio with glycerol administration. Glycerin has yielded significant improvements in beef cattle performance (Parsons et al., 2009) when administered at low levels, and it is conceivable that the disproportionate improvement in performance is due to impact of glycerin on ruminal fermentation. Availability of information pertaining to the effects of crude glycerin on ruminal fermentation and diet digestibility in high-concentrate diets is limited.

Our objectives were to determine if glycerol addition to feedlot diets influences ruminal fermentation patterns, pH, and concentrations of VFA, ammonia, and lactate, and to evaluate its impact on apparent total tract digestibility of feedlot diets.

## **MATERIALS AND METHODS**

Procedures for this experiment were approved by the Kansas State University Institutional Animal Care and Use Committee. Crossbred steers ( $n = 9$ ;  $624 \pm 80$  kg) fitted with ruminal cannulas (Bar Diamond Inc., Parma, ID; dorsal sac) were used in a randomized complete block design experiment utilizing three treatments and three periods. Treatments consisted of steam-flaked corn diets containing 0, 2, and 4% crude glycerin (DM basis). Steers were allowed *ad libitum* access to finishing diets fed once daily at 0800 h. Periods consisted of 13 d, with 10 d for adaptation and 3 d for sample collection. Cattle were housed in individual slatted floor metabolism stalls measuring  $1.5 \times 3$  m and equipped with automatic drinking fountains at the rear and fence line feed bunks at the front of each pen. Determination of the amount of feed offered was made approximately one h before daily delivery of rations. Diets were mixed in a stationary mixer (Davis Manufacturing Co., Bonner Springs, KS) and weighed into individual tubs. Feed refusals were taken on d 11 through 14; orts were weighed and dried at  $55^\circ$  C for 48 h. The weight of refused feed was subtracted from the total feed delivered during the 3-d sampling period to estimate DMI. Feed ingredients were sampled weekly and analyzed for CP, crude fat, calcium, phosphorus, NDF, and starch. Average composition over the feeding period for each ingredient was used to calculate diet composition in Table 1.

Ruminal digesta was collected at the following times relative to feeding: d 1 at 0, 6, 12, 18 h; d 2 at 2, 8, 14, 20 h; and d 3 at 4, 10, 16, 22 h. At each collection point, ruminal digesta

was strained through eight layers of cheesecloth. A portable pH meter (model 230, Thermo Orion, Waltham, MA) measured the pH of the strained rumen fluid. Strained ruminal fluid (4 mL) was combined with 1 mL of 25% (wt/vol) metaphosphoric acid and then frozen at -20°C. Acidified ruminal fluid samples were later thawed and centrifuged at 15,000 x g for 15 min at 4°C, and a portion of the supernatant fluid was analyzed for lactate and VFA by gas chromatography (Hewlett-Packard 5890A, Hewlett-Packard, Palo Alto, CA; 2 m × 2 mm column, Supelco B-DA 80/120 4% Carbowax 20-m column packing, Supelco, Bellefonte, PA), with nitrogen as the carrier gas, a flow rate of 24 mL/min, and a column temperature of 175°C. A portion of the supernatant was analyzed for ammonia concentrations with a Technicon Autoanalyzer III (Bran and Luebbe, Elmsford, NY) in accordance with procedures described by Broderick and Kang (1980).

Chromic oxide (10 g/d) was dosed daily as an indigestible marker d 3 through 10 using a gelatin capsule (Torpac Inc., Fairfield, NJ) and placed in the rumen prior to feeding each day to estimate total fecal output. Fecal samples were taken from each steer coinciding with ruminal fluid sampling. Diet fecal samples were oven dried (Fisher Scientific, Hanover Park, IL) at 55°C for 2 d, air-equilibrated, and ground through a Wiley mill (Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen. Approximately 5 g of each dry ground fecal sample were pooled within each collection period per animal and stored for chemical analysis. Ground samples of experimental diets and fecal samples were dried at 105°C for 24 h to determine DM. Organic matter was determined by ashing the dried samples at 600°C for 2 h in a muffle furnace (Thermo Fisher Scientific, Hanover Park, IL). Starch content of the diet and fecal samples dried at 55°C for 2 d were determined according to the methods described by Herrera-Saldana and Huber (1989) using a Technicon Autoanalyzer III to measure free glucose (Gochman and Schmitz,

1972). NDF was determined by an Ankom 200 Fiber Analyzer (Ankom Technologies, Macedon, NY) with the procedures of Van Soest et al. (1991). Heat-stable  $\alpha$ -amylase (Ankom Technologies) was added to fecal samples (dried at 55°C for 2 d) to remove residual starch for NDF determination. Crude fat of diets and fecal samples was measured in accordance with AOAC official method 920.39 (AOAC, 1995). Chromic oxide concentrations of fecal samples were determined by atomic absorption spectrophotometry with an acetylene/air flame (Perkin-Elmer 3110, Perkin-Elmer Norwalk, CT) according to the methods of Williams et al. (1962).

### ***Calculations and Statistical Analysis***

Chromic oxide was used as an indigestible marker to estimate total tract digestibility. Total fecal output for each steer within each period was estimated by dividing chromic oxide consumed (g/d) by chromic oxide concentrations in the feces (g/kg of DM). Apparent total tract digestibilities of DM, OM, NDF, starch, and ether extract were estimated using the following formula:  $[(\text{Intake of nutrient} - \text{fecal output of nutrient})/\text{intake of nutrient}] \times 100$ .

Apparent total tract digestibilities were statistically analyzed using the MIXED procedure (SAS Inst. Inc., Cary NC). Animal served as the experimental unit, and random effects include period, animal, and interactions. The model statement included treatment. Orthogonal contrasts were used to determine linear and quadratic effects of glycerin. Treatment means were computed using LSMEANS option. The MIXED procedure of SAS was performed for VFA profiles, pH, and ammonia concentrations. Animal served as the experimental unit. The model statement included treatment, time post-feeding, and treatment  $\times$  time post-feeding. Random effects included period, pen, and interactions. Orthogonal contrasts were used to determine the linear and quadratic effects of glycerin.

## **Results and Discussion**

We observed no treatment  $\times$  time post-feeding interactions ( $P > 0.2$ ). Glycerin had no effect on DMI (Table 2). These results are similar to those of Mach et al. (2009), who noted no differences in dietary intake when glycerin was included up to 12% (DM basis) of the diet and fed to Holstein bulls. Other research feeding heifers suggests that increasing glycerin concentrations in the diet will decrease dietary feed intake. Parsons et al. (2009) observed no differences in DMI when glycerin was fed at 0 or 2% of the diet, but DMI decreased when concentrations of glycerin increased to 4, 8, 12, and 16 % (linear,  $P < 0.001$ ; quadratic,  $P = 0.014$ ). Glycerin concentrations for the current trial were potentially low enough to have no effect on DMI. Intakes of OM, CP, crude fat, starch, and NDF were similar across treatments (linear  $P > 0.32$ ; quadratic,  $P > 0.91$ ). A reduction in starch intake in cannulated steers by 0.7 kg/d was observed when cattle were fed 15% glycerol (Schröder and Südekum, 2007). Feeding glycerin had no effect on apparent total tract digestibilities of DM, OM, starch, CP, or crude fat, but tended to (linear,  $P = 0.12$ ) decrease NDF digestibilities. Previous research has suggested that glycerin affects cellulolytic activity in the rumen. Decreases in cellulose degradation by cellulolytic bacteria and cellulolytic fungi were observed *in vitro* when media contained 0.5% and 5% glycerin, respectively (Roger et al., 1992). Supporting this, glycerin reduced IVDMD of oat hay and carboxymethyl-cellulose (Paggi et al., 2004). Decreases in cellulolytic activity could explain the decrease in NDF digestion observed in our study. In contrast, Hess et al. (2008) added up to 15% glycerin *in vitro* without affecting fiber digestion, with the exception of cool-season grasses. Differences in digestibility could be attributed to ability of microorganisms to adapt to glycerin feeding, as disappearance rates of glycerin may increase with increasing days on feed (Krehbiel, 2008). Fiber digestibility could potentially improve over time due to adaptation of ruminal microbes. Though, changes in fiber digestion are of limited concern in



grain-based feedlot diets because fiber constitutes only a small component of these diets. However, concerns arise when cattle are fed forage-based step-up diets or diets containing other by-products, which can be higher in fiber. Some research has suggested that glycerin also may impact protein metabolism negatively. Adding glycerin at 50, 100, 200, or 300 mM to medium reduced proteolytic activity by 20% compared to medium without glycerin (Paggi et al., 1999). Changes in apparent total tract digestibility of protein were not observed in our trial (linear,  $P > 0.57$ ) though this admittedly is a poor indication of protein status. Difference in digestibilities of diet components could be attributed to the differences in composition of crude glycerin or the fat source which the glycerin is manufactured from.

Average pH values were 5.61, 5.67 and 5.73 when glycerin concentrations were 0, 2, and 4, respectively (Figure 1; linear  $P < 0.05$ ). Ruminal pH in dairy cows fed 0, 0.43, or 0.86 kg/d glycerin were 6.91, 6.89, and 6.61, respectively ( $P < 0.13$ ; DeFrain et al., 2004). Schröder and Südekum (1999) noted that postprandial pH actually was greater in steers fed glycerin diets. Differences in roughage level of the diets could explain the differences in pH. Roughage concentrations for DeFrain et al (2004) and Schröder and Südekum (1999) were greater than 40% of the diet (DM), whereas our diets contained only 5.9% roughage. Glycerin is rapidly fermented, which could decrease pH in high roughage diets with high pH values and may increase pH in high concentrate diets with pH below 6.0.

Feeding glycerin tended (linear,  $P = 0.06$ ) to decrease acetate concentrations (Figure 2). Propionate concentrations were not affected by glycerin (Figure 3; linear,  $P < 0.5$ ; quadratic,  $P < 0.3$ ). Likewise, Rémond et al. (1993) observed significant ( $P < 0.05$ ) reductions in acetic acid concentrations in cows consuming a maize silage diet and supplemented with 0, 0.2, or 1.2 kg/d. Previous studies have reported increases in propionate concentrations when animals were

supplemented with glycerin (Czerkawski and Breckenridge, 1972; DeFrain et al., 2004; and Trabue et al., 2007). Butyrate concentrations decreased linearly ( $P < 0.05$ ) as glycerin levels increased from 0 to 4% of diet DM (Figure 4). In contrast, Schröder and Südekum (1999) reported higher butyric acid concentrations in glycerin fed cattle, with peak levels at 3 h post feeding ( $P < 0.05$ ). As well, Rémond et al. (1993), reported increased production of propionate and butyrate at the expense of acetate. Molar concentrations of valerate also were lower in cattle fed glycerin (Figure 5; linear,  $P < 0.01$ ; quadratic,  $P > 0.5$ ). Reductions in valerate concentrations maybe related to changes in protein catabolism. Total VFA concentrations were similar across all treatments (Table 3; linear,  $P < 0.23$ ; quadratic,  $P < 0.31$ ). Likewise, no difference in VFA concentrations were noted for Holstein bulls fed up to 12% (DM) crude glycerin (Mach et al., 2009). DeFrain et al. (2004) reported total VFA concentrations of dairy cows postpartum were 56.2, 70.2, and 61.4 when fed 0, 0.43, or 0.86 kg/d glycerin, respectively ( $P = 0.06$ ). Adding glycerin to fermenters containing cellulose substrates increased VFA concentrations during the first 6 h ( $P < 0.01$ ), but had no effect on VFA production when added to fermenters containing starch as substrate (Rémond et al., 1993). Glycerin's effect on increasing total VFA concentrations is observed in diets containing large amounts of cellulose, while changes in high- concentrate feedlot diets are less apparent. Isobutyrate and isovalerate were not affected by increasing amounts of glycerin in the diet. Ruminal ammonia concentrations also were unchanged as dietary glycerin concentrations increased (linear,  $P = 0.72$ ). Supporting this, DeFrain et al. (2004) reported no differences in ruminal ammonia concentrations in postpartum dairy cows supplemented glycerin. Our results suggest that feeding glycerin may have deleterious effects on fiber digestion. While this may have limited impact on traditional

grain-based finishing feedlot diets, it is conceivable that digestion of diets containing greater quantities of forage or grain byproducts would be impacted to a greater extent.

**Table 3-1 Composition of finishing diets containing 0, 2, or 4% crude glycerin fed to ruminally cannulated steers**

Item	Crude Glycerin, %		
	0	4	8
Ingredient, %			
Steam-flaked corn	82.6	80.2	77.8
Corn steep liquor	5.7	5.7	5.7
Ground alfalfa hay	5.9	5.9	5.9
Crude Glycerin <sup>1</sup>	0.0	2.0	4.0
Soybean meal	0.4	0.8	1.2
Urea	1.2	1.2	1.2
Supplement <sup>2</sup>	2.0	2.0	2.0
Feed additive premix <sup>3</sup>	2.2	2.2	2.2
Nutrient, %			
DM	81.0	81.2	81.3
OM	94.1	94.0	93.9
NDF	10.5	10.3	10.1
Starch	59.0	57.4	55.7
CP	13.9	13.9	13.8
Crude fat	3.5	3.4	3.5
Ca	0.66	0.67	0.67
P	0.32	0.32	0.31

<sup>1</sup>Methanol content of crude glycerin < 0.01%

<sup>2</sup>Formulated to provide 0.1 mg/kg Co; 10 mg/kg Cu; 0.6 mg/kg I; 60 mg/kg Mn; 0.25 mg/kg Se; 60 mg/kg Zn; 1.0% K; 2,640 IU/kg vitamin A; and 22 IU/kg vitamin E

<sup>3</sup>Provided 300 mg of monensin (Elanco, Indianapolis, IN) and 90 mg of tylosin (Elanco) per animal daily in a ground corn carrier

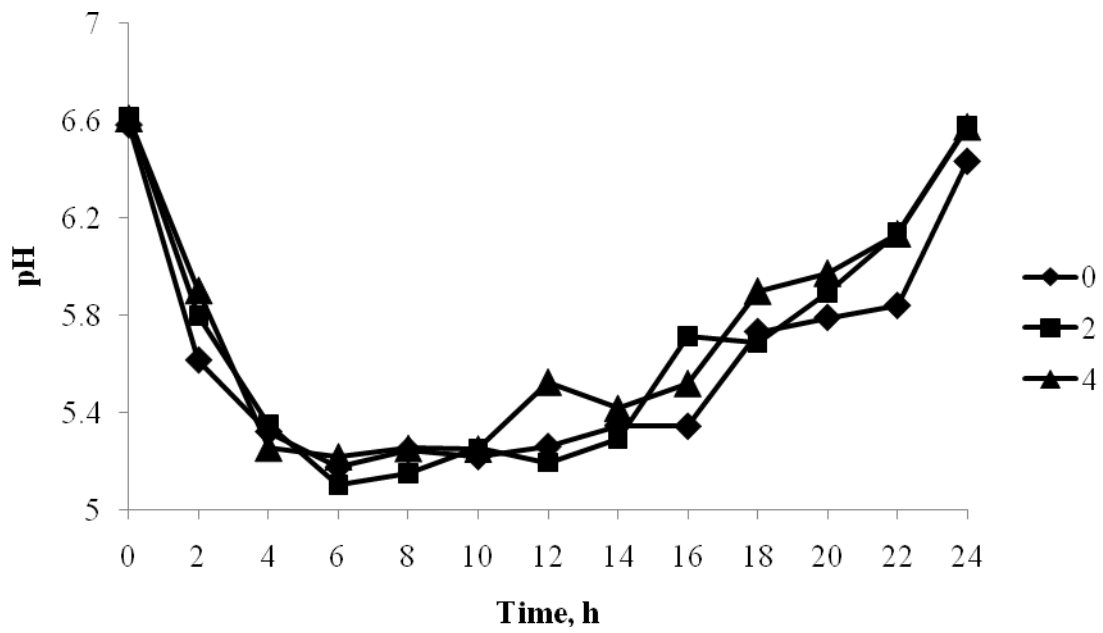
**Table 3-2 Digestion characteristics for ruminally cannulated steers fed crude glycerin**

Item	Crude Glycerin			SEM	Contrast P-values	
	0	2	4		Linear	Quadratic
Intake kg/d						
DM	7.98	8.01	8.05	0.40	0.86	0.99
OM	7.50	7.53	7.55	0.38	0.89	0.91
Starch	4.72	4.60	4.48	0.23	0.32	0.99
NDF	0.84	0.83	0.81	0.04	0.58	0.91
CP	1.11	1.11	1.11	0.06	0.93	0.99
Crude fat	0.28	0.27	0.27	0.01	0.33	0.99
Fecal excretion, kg						
DM	1.21	1.27	1.28	0.17	0.51	0.76
OM	0.97	0.99	1.03	0.13	0.46	0.90
Starch	0.018	0.015	0.019	0.005	0.77	0.40
NDF	0.42	0.46	0.47	0.07	0.12	0.63
CP	0.24	0.23	0.25	0.03	0.64	0.58
Crude fat	0.029	0.026	0.027	0.005	0.65	0.68
Apparent total tract digestibility, %						
DM	84.9	84.2	84.2	1.70	0.60	0.76
OM	87.1	86.9	86.4	1.36	0.52	0.91
Starch	99.7	99.6	99.6	0.10	0.21	0.96
NDF	49.9	45.8	43.4	6.0	0.13	0.82
CP	79.0	79.0	79.0	2.24	0.57	0.70
Crude fat	90.0	90.1	90.0	1.80	0.93	0.87

**Table 3-3 Ruminal VFA and ammonia concentration in ruminally cannulated steers fed crude glycerin at 0, 2, or 4%**

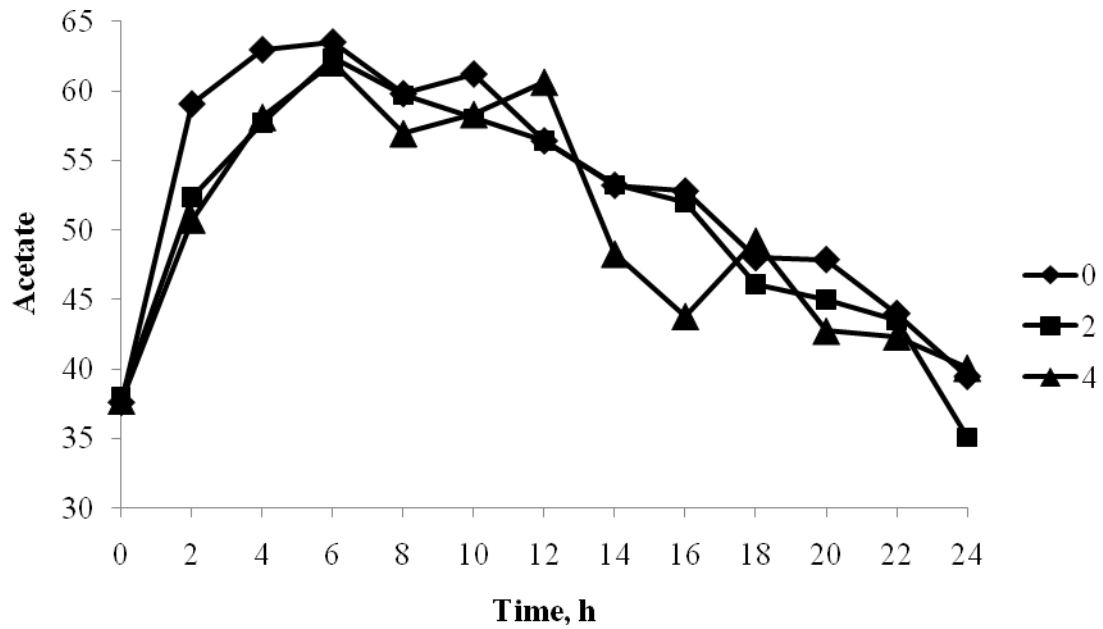
Item	Glycerin			SEM	P-values	
	0	2	4		Linear	Quadratic
A:P Ratio	1.16	1.20	1.14	0.14	0.68	0.25
Isobutyrate, mM	0.88	0.85	0.84	0.05	0.32	0.92
Isovalerate, mM	2.25	1.85	2.35	0.37	0.80	0.20
Total VFA, mM	120.2	115.6	116.4	4.7	0.23	0.31
Ammonia, mM	7.99	7.80	7.67	1.33	0.72	0.97

**Figure 3-1 Ruminal pH in ruminally cannulated steers fed diets containing 0, 2, or 4% crude glycerin.**



SEM = 0.12; Linear ( $P = 0.05$ ); Quadratic ( $P = 0.94$ ); Hour Effect ( $P < 0.0001$ );  
Treatment  $\times$  Time Effect ( $P = 0.2655$ )

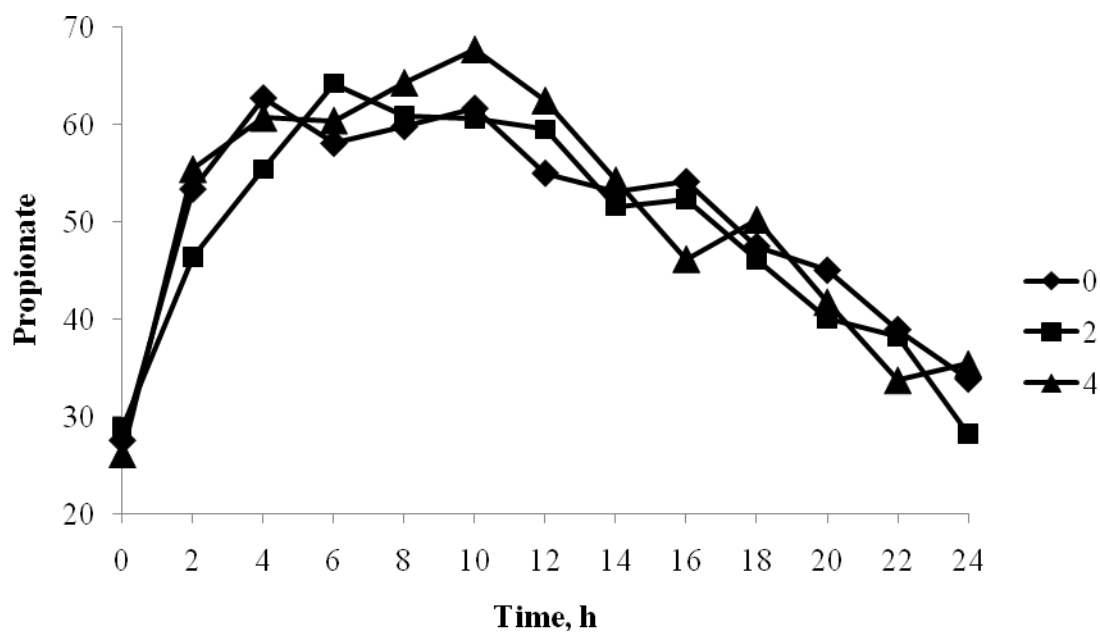
**Figure 3-2** Ruminal acetate concentrations in ruminally cannulated steers fed diets containing 0, 2, or 4% crude glycerin.



SEM = 1.60; Linear ( $P = 0.06$ ); Quadratic ( $P > 0.50$ ); Hour Effect ( $P < 0.001$ );  
Treatment  $\times$  Time Effect ( $P = 0.7025$ ).

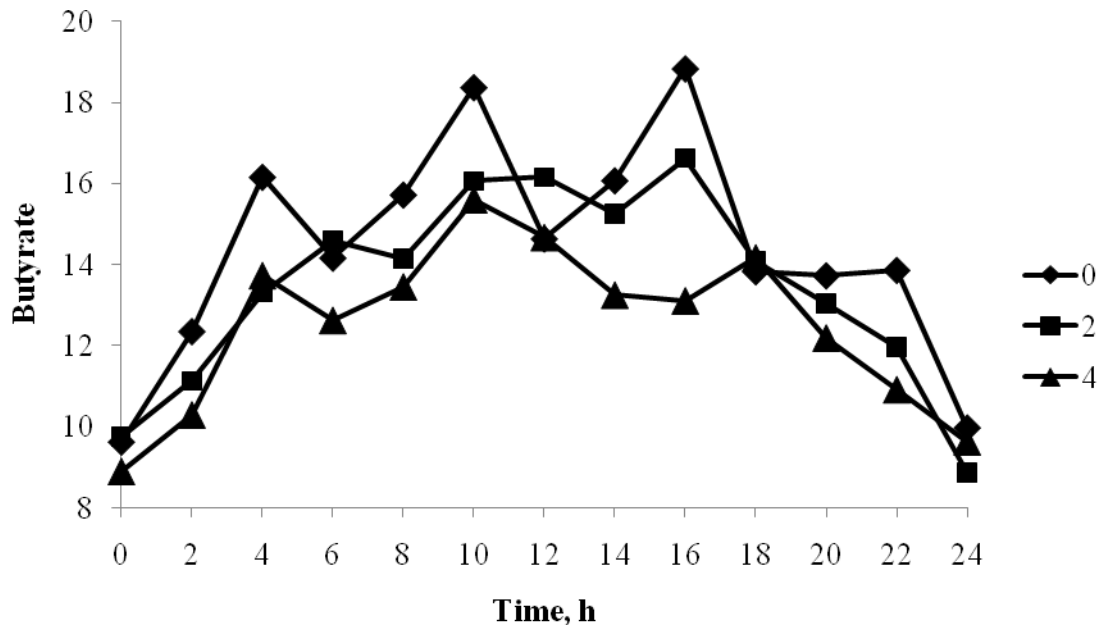


**Figure 3-3** Ruminal propionate concentrations in ruminally cannulated steers fed diets containing 0, 2, or 4% crude glycerin.



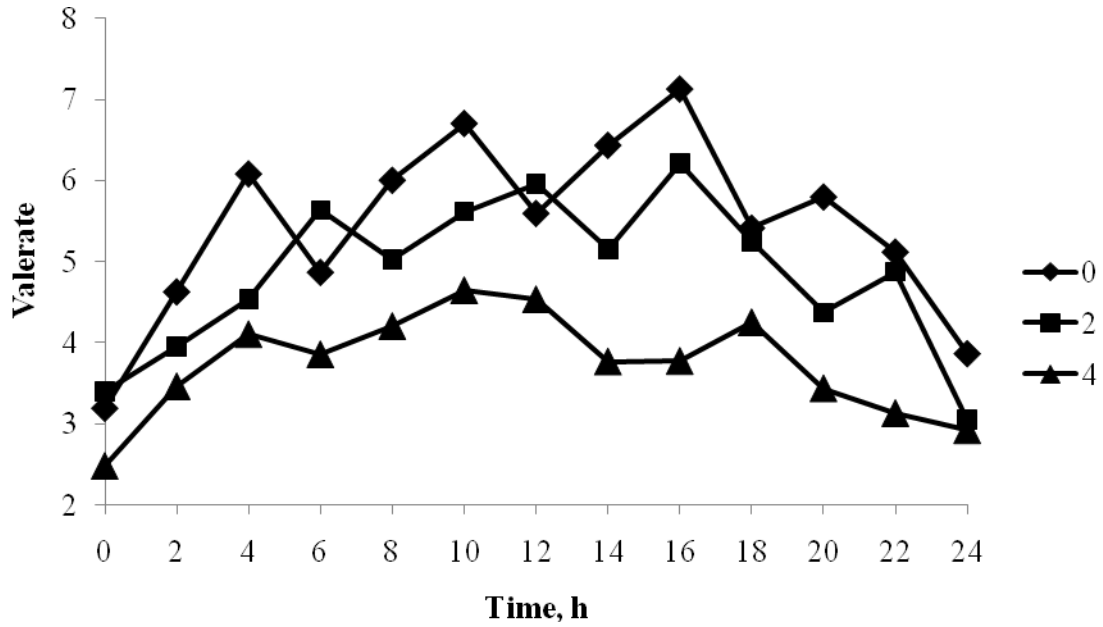
SEM = 3.5; Linear ( $P > 0.5$ ); Quadratic ( $P > 0.3$ ); Hour Effect ( $P < 0.001$ );  
Treatment  $\times$  Time Effect ( $P = 0.7431$ ).

**Figure 3-4** Ruminal butyrate concentrations in ruminally cannulated steers fed diets containing 0, 2, or 4% crude glycerin.



SEM = 1.05; Linear ( $P < 0.05$ ); Quadratic ( $P > 0.5$ ); Hour Effect ( $P < 0.001$ );  
Treatment  $\times$  Time Effect ( $P = 0.5290$ ).

**Figure 3-5** Ruminal valerate concentrations in ruminally cannulated steers fed diets containing 0, 2, or 4% crude glycerin.



SEM = 0.73; Linear ( $P < 0.01$ ); Quadratic ( $P > 0.5$ ); Hour Effect ( $P < 0.001$ );  
Treatment  $\times$  Time Effect ( $P = 0.2890$ ).

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**APPENDIX A: Effect of extended Zilpaterol hydrochloride  
withdrawal on performance and carcass traits of finishing heifers**

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## ABSTRACT

Crossbred heifers (n = 450; 465 kg ± 27.2) were used in a randomized complete block experiment to evaluate withdrawal times for zilpaterol hydrochloride. Heifers were blocked into heavy and light BW groups and randomly allocated within block to feedlot pens containing 7 to 10 heifers each, with 9 pens/treatment. Treatments were arranged as a 2 × 3 factorial, with factors consisting of zilpaterol-hydrochloride (**Z**) fed at 0 or 8.33 mg/kg DM for 20d and withdrawal times (**W**) of 3, 10, or 17 d prior to harvest. Heifers were implanted with Finaplix-H and fed flaked corn finishing diets *ad libitum* once daily, providing 300 mg monensin, 90 mg tylosin, and 0.5 mg melengestrol acetate per animal daily. With the exception of yield grade, there were no significant Z × W interactions ( $P > 0.10$ ). For yield grade, Z × W interaction occurred because Z improved USDA yield grade after 3 and 10 d of withdrawal, but no differences were detected by 17 d. Zilpaterol did not affect DMI, ADG, or gain efficiency ( $P > 0.10$ ), but increased HCW, dressing percentage, and LM area ( $P < 0.01$ ) and decreased marbling scores ( $P < 0.01$ ). Zilpaterol increased HCW by 12.8, 7.7, and 5.2 kg after 3, 10, and 17 d of withdrawal, respectively. Marbling scores were 457, 466, and 459 in control cattle, and 401, 445, and 442 in Z cattle after 3, 10, and 17 d of withdrawal, respectively. Final BW, HCW, and marbling scores ( $P < 0.04$ ) increased with longer W, and back fat and KPH decreased ( $P < 0.03$ ). Whole loins were collected from 15 randomly selected cattle per treatment in each block and wet aged in vacuum bags for 7, 14, or 21d. Zilpaterol increased shear force in steaks by 0.54 kg ( $P < 0.001$ ), but shear force declined linearly with additional aging ( $P < 0.01$ ), yielding loin steaks with acceptable shear-force after 14 or 21 d of aging.

**Key words:** zilpaterol, shear force, performance



## INTRODUCTION

Zilpaterol hydrochloride (**Z**) is an orally active  $\beta$ -adrenergic agonist ( **$\beta$ AA**) growth promoting compound utilized in Mexico and South Africa for the previous 12 y (Avendaño-Reyes et al., 2006). Approval for the use of Z in the United States was granted in August of 2006 to be fed at 8.33 mg/kg (DM basis) for the final 20 to 40 days on feed, with a minimum of a 3-d withdrawal period (FDA, 2006). Beta-adrenergic agonists elicit response by activating beta-receptors in muscle and fat tissues, stimulating protein accretion and lipolysis, while decreasing lipogenesis (Mersmann, 1998). Feeding these compounds during the final phase of feeding increases protein and decreases fat in the carcass (Leheska et al., 2009). Zilpaterol hydrochloride has been observed to increase ADG, G:F, HCW, and improve dressing percentage (Hilton et al., 2009; Montgomery et al., 2009; Vasconcelos et al., 2008). Feeding Z also has been noted to increase shear-force values. Increases of 20 to 28% in Warner-Bratzler shear force (WBSF) were observed when South African steers were fed Z for the final 30 to 50 d (Strydom and Nel, 1996; Strydom et al., 2002). Other beta agonists, such as clenbuterol, cimaterol, and ractopamine, have increased shear-force (Moloney et al., 1990; Schiavetta et al., 1990; Schroeder et al., 2003; Vestergaard et al., 1994). Our objective in this study was to determine if extended withdrawal of Z would ameliorate negative effects on marbling score and shear force without sacrificing improvements in carcass weight.

## MATERIALS AND METHODS

Procedures for this experiment were approved by the Kansas State University Institutional Animal Care and Use Committee. Crossbred heifers (n = 450 ; 465 kg  $\pm$  27.2 ) were blocked into heavy and light blocks. Two animals were removed from trial for injuries unrelated to treatment. Within weight block, cattle were stratified by BW and allocated

randomly to feedlot pens containing 7 to 10 heifers each, with 9 pens/treatment. Three replicates of animals (two replicates from the light block and 1 replicate from the heavy block) were housed on concrete-surfaced pens (36.5 m<sup>2</sup>) with roofs covering half the pen and the entire feed bunk; 2 replicates were housed on uncovered concrete-surfaced pens (4.27 m × 6.10 m); and 4 replicates were housed on uncovered, dirt surfaced pens (278 m<sup>2</sup>) with a 3.66 m concrete apron extending from the bunk. Treatments were arranged as a 2 × 3 factorial, with factors consisting of zilpaterol hydrochloride (Z) fed at 0 or 8.33 mg/kg DM for 20 d and withdrawal times (W) of 3, 10, or 17 d. Heifers were implanted with Finaplix-H and fed a 94% concentrate diet with 6% alfalfa hay (Table 1). Diets were formulated to provide 300 mg/d monensin, 90 mg/d tylosin (Elanco Animal Health, Greenfield, IN), and 0.5 mg/d of melengestrol acetate (Pfizer Animal Health, Exton, PA). Cattle were fed once daily (afternoon) *ad libitum*. Heifers in the heavy block were fed for 48, 55, or 62 d, while the heifers in the light block were fed for 84, 91, or 98 d. Cattle were weighed and transported 450 km to a commercial abattoir, where carcass data were collected. Hot carcass weights and liver scores were obtained at the time of harvest. Longissimus muscle area; 12<sup>th</sup>-rib fat thickness; marbling score; KPH; incidence of dark cutting beef; and USDA quality and yield grades were collected following a 48-h chill period. Whole loins were collected from fifteen carcasses per treatment each of the six harvest days. Loin samples from the heavy block consisted of 8 grading USDA Select and 7 grading USDA Choice, and the light block consisted of 7 grading USDA Select and 8 grading USDA Choice for each harvest day. Loins were vacuum packaged and wet aged for 7, 14, or 21 d. At the end of each aging time point, one steak was cut from the anterior end of each loin, vacuum packaged, and frozen. The remainder of each loin was repackaged in CryoVac bags and stored at 4° C until the end of the next aging period. Frozen steaks were tempered for 24 h prior to cooking at

approximately 4° C. Steaks were cooked in a forced-air convection oven (model DFG-102 CH3, G. S. Blodgett Co., Burlington, VT) set at 163° C. Steaks were turned at an internal temperature of 40° C and cooked to an internal temperature of 70° C, as monitored with thermocouples placed in the approximate geometric center of each steak. Eight cores (1.27 cm in diameter) were drilled parallel to the muscle fiber using a mechanical coring device from eight standardized locations. Each core sample was analyzed for shear-force value using an Instron (model 5569; Instron, Norwood MA) machine. Peak force (kg) was recorded for each core, and the 8 cores were used to calculate an average shear force value for each steak.

### ***Statistical Analysis***

Performance and carcass data were analyzed as a randomized complete block design using the Mixed model procedure of SAS (SAS Inst. INC., Cary, NC). Pen served as the experimental unit. Model effects included zilpaterol-hydrochloride level, withdrawal time, and the interaction between zilpaterol-hydrochloride level and withdrawal time. The random effect was block. Treatment means were computed with the LSMEANS option.

Shear-force data were analyzed as a randomized complete block design using the Mixed model procedure of SAS (SAS Inst. INC., Cary, NC). Pen served as the experimental unit. Model effects included zilpaterol-hydrochloride level, withdrawal time, wet aging time, and the interactions. Random effects included block and the block by zilpaterol-hydrochloride by withdrawal time interaction. Treatment means were computed with the LSMEANS option.

## **RESULTS**

### ***Feedlot Performance***

Performance results are presented in Table 2. ADG over the entire feeding period was not affected by feeding Z ( $P > 0.54$ ) or increasing withdrawal times ( $P > 0.75$ ). Similarly, Holland

et al. (2010) saw no improvement in ADG ( $P \geq 0.21$ ) in steers, but noted that zilpaterol tended to ( $P = 0.09$ ) increased carcass-adjusted ADG. Dry matter intakes over the entire feeding period were 8.74 kg/d for control heifers and 8.56 kg/d for Z fed heifers and were not different ( $P > 0.15$ ). Decreases in DMI have been noted when Z is fed to both steers and heifers (Avendaño-Reyes et al., 2006; Holland et al., 2009; and Montgomery et al., 2009). Zilpaterol-treated cattle tended to be more efficient than control heifers ( $P = 0.11$ ). Efficiency improved by an average 4.4% when Z was fed. Montgomery et al. (2009) noted that during the 20 d of zilpaterol feeding and a 5 d withdrawal a 21% and 28% improvement in G:F when zilpaterol was fed to heifers and steers, respectively. As expected, increasing withdrawal times from 3, 10, to 17 d significantly increased final BW ( $P < 0.01$ ) because cattle had increased DOF. Holland et al., (2009) reported that Z had no effect on final body weight ( $P \geq 0.14$ ) of steers, but linear increases ( $P < 0.01$ ) in final BW were observed as withdrawal times increased. Robles-Estrada et al., (2009) reported no differences in final BW in heifers when zilpaterol was fed for the final 30 DOF with a 3-d withdrawal time ( $P > 0.77$ ). Feeding Z has limited effects on ADG or final BW.

### ***Carcass Characteristics***

Compared to heifers in the control groups HCW were increased by 12.8, 7.7, and 5.2 kg when heifers were fed Z and had withdrawal periods of 3, 10, and 17 d, respectively (Table 3). Holland et al. (2009) reported an interaction between zilpaterol and withdrawal period in steers, with increases in HCW of 14, 17, 5, and 6 kg when withdrawal d were 3, 10, 17, and 24, respectively. Increases in HCW have been consistently observed with Z feeding (Elam et al., 2009; Montgomery et al., 2009; and Vasconcelos et al., 2008). Increasing withdrawal times will allow some marketing flexibility, but it is unclear at what withdrawal time zilpaterol feeding has a negative return on investment.

Zilpaterol improved dressing percentage by 1.6, 1.22, and 1.04% with withdrawal times of 3, 10, and 17, respectively. This increase in dressing percentage is consistent with previous research results (Vasconcelos et al. 2008; Holland et al. 2009; and Montgomery et al. 2009). Significant ( $P < 0.01$ ) increases in longissimus muscle area occurred when Z was fed. The magnitude of increase in LM area from Z became less as withdrawal time increases. Zilpaterol feeding had no effect on 12<sup>th</sup>-rib fat thickness, KPH, or the percentage of liver accesses ( $P > 0.16$ ). Withdrawal from Z feeding impacted KPH ( $P < 0.01$ ); lower values were recorded at the 10-d withdrawal, but we suspect this may be due to differences in personnel estimating KPH, since one person estimated KPH for withdrawal times of 3 and 17 d, and a second person estimated KPH on d 10. Also, withdrawal time affected 12<sup>th</sup>-rib fat thickness ( $P < 0.05$ ); as withdrawal times increased, so did fat thickness in the control heifers, but the Z-fed heifers were leaner at 10-d withdrawal when compared to 3 and 17 d.

A significant  $Z \times W$  interaction for USDA yield grades was observed. Zilpaterol significantly improved average USDA yield grade ( $P < 0.01$ ) when withdrawal times were 3 and 10 d, but had no effect by 17 d. Improvements in USDA yield grade with respect to 3-d withdrawal times are consistent with previous reports (Elam et al., 2009; Montgomery et al., 2009; Vasconcelos et al., 2008). Zilpaterol decreased marbling scores ( $P < 0.01$ ) but increasing withdrawal times alleviated some of these effects on marbling scores ( $P < 0.05$ ). Zilpaterol decreased the percentage of cattle grading USDA Prime and Choice and increased the percentage of cattle grading USDA Select and Standard ( $P < 0.08$ ). Increasing withdrawal times to 10 and 17 d resulted in greater percentages of cattle grading USDA Choice and reduced percentage of carcasses grading USDA Select. Similarly, Holland et al. (2009) noted that increased withdrawal times in steers increased the percentage of cattle grading USDA Choice.

### ***Shear Force***

Shear force values are presented in Table 3. Feeding Z increases shear-force values ( $P < 0.01$ ) when compared to control steaks. Increases in shear force values with supplementation of  $\beta$ -agonists have been noted previously (Shook et al., 2009; Strydom et al., 2002). Increasing withdrawal time did not improve tenderness ( $P > 0.31$ ). Shook et al. (2009) reported higher shear force values when animals had withdrawal times of 3 and 24 d, while steaks from animals with 10 and 17 d had the lowest shear force values. An interaction between Z  $\times$  Aging ( $P < 0.05$ ) was observed as wet aging time increased shear-force values of steaks from heifers fed Z improved more than their control counterpart. Increasing the length of wet aging significantly improved shear force values ( $P < 0.01$ ) and acceptable shear-force values are obtained by wet aging Z steaks for 14 d or greater. Steaks with shear-force value less than 3.9 kg have a 68% chance of being acceptable in tenderness by consumers (Platter et al. 2003). In agreement with our results, Strydom et al. (2009) indicated that aging steaks for 14 d or longer will reduce shear force values. Overall, Z has limited or no effects on live feedlot performance, but improves HCW, dressing percentage, USDA yield grade, and LM area in finishing heifers. Increasing withdrawal times can improve marbling scores but at the expense of HCW improvements. Improvements in tenderness are not achieved by increasing Z withdrawal times.

**Table A-1 Diet composition, 100% DM basis**

<b>Ingredient</b>	<b>Treatments</b>	
	<b>Control</b>	<b>Zilmax</b>
Steam-flaked corn	80.48	80.46
Alfalfa hay	6.0	6.0
Corn steep liquor	8.0	8.0
Control premix <sup>1</sup>	3.29	--
Zilpaterol premix <sup>1</sup>	--	3.31
Feed additive premix <sup>2</sup>	2.23	2.23
Nutrients		
DM	80.2	80.2
CP	14.8	14.9
Ca	0.65	0.66
P	0.33	0.33

<sup>1</sup>Formulated to provide 0.1 mg/kg Co; 10 mg/kg Cu; 0.6 mg/kg I; 60 mg/kg Mn; 0.25 mg/kg Se; 60 mg/kg Zn; 1.0% K; 2,640 IU/kg vitamin A; and 22 IU/kg vitamin E

<sup>2</sup>Feed additive premix was formulated to provide 300 mg monensin, 90 mg tylosin, and 0.5 mg melengestrol acetate per heifer daily in a ground corn carrier.

**Table A-2 Effects of zilpaterol-hydrochloride and withdrawal time on feedlot heifer performance**

Item	Treatment <sup>1</sup>						SEM	<i>P</i> -Values <sup>2</sup>		
	3		10		17			Z	W	Z×W
	C	Z	C	Z	C	Z				
Initial BW, kg	466.6	469.8	468.3	468.1	468.3	467.9	26.28	0.68	0.99	0.73
Final BW, kg <sup>3</sup>	551.2	557.4	562.8	564.0	575.1	573.8	8.29	0.64	< 0.02	0.77
ADG, kg/d	1.27	1.34	1.32	1.35	1.35	1.35	0.08	0.54	0.75	0.81
DMI, kg/d	8.64	8.61	8.83	8.45	8.75	8.63	0.32	0.16	0.91	0.50
G:F	0.1470	0.1552	0.1491	0.1589	0.1538	0.1557	0.005	0.11	0.74	0.71

<sup>1</sup>Calculated by multiplying final body weight by 96 percent

<sup>2</sup>Treatment: C = Control and Z = zilpaterol-hydrochloride fed cattle

<sup>3</sup>*P*-Values: Z = zilpaterol-hydrochloride; W = withdrawal time; Z×W = interaction



**Table A-3 Effects of Zilpaterol-hydrochloride and withdrawal time on carcass characteristics**

Withdrawal Day	Treatment <sup>1</sup>						SEM	P-Values <sup>2</sup>		
	C		Z		C			Z	W	Z×W
	3	3	10	10	17	17				
HCW, kg	351.5	364.3	361.9	369.6	366.5	371.7	3.43	<0.04	0.01	0.54
Dressed yield, %	63.78	65.38	64.34	65.56	63.74	64.78	0.72	<0.01	0.13	0.70
LM area, cm <sup>2</sup>	88.45	95.36	87.73	94.33	87.22	91.45	3.79	<0.01	0.24	0.64
USDA yield grade										
Yield grade 1, %	13.2	22.9	11.5	27.3	18.0	8.9	5.0	0.17	0.45	0.04
Yield grade 2, %	45.5	42.8	40.2	46.3	31.7	47.9	8.0	0.17	0.73	0.27
Yield grade 3, %	38.9	29.7	38.8	25.1	47.2	39.0	8.0	0.05	0.19	0.90
Yield grade 4 & 5, %	2.4	4.6	9.5	1.3	3.1	4.2	4.0	0.41	0.69	0.07
Average yield grade	2.30	2.16	2.46	2.00	2.37	2.38	0.18	0.017	0.23	0.056
USDA quality grade										
Prime, %	5.0	0.1	1.7	0.1	3.2	0.1	2.0	0.01	0.53	0.53
Choice, %	64.0	44.3	75.2	71.7	69.1	67.4	5.0	0.07	0.01	0.20
Select, %	31.0	54.0	23.1	28.2	27.7	29.7	5.0	0.03	0.01	0.11
Standard, %	0	1.6	0	0	0	2.8	1.0	0.08	0.38	0.38
Marbling score <sup>3</sup>	457	404	466	445	459	442	11	0.01	0.04	0.14
S.C. fat, cm	1.08	1.11	1.16	1.01	1.29	1.18	0.15	0.16	0.03	0.34
KPH, %	2.13	2.25	2.00	1.98	2.24	2.22	0.06	0.57	<0.01	0.33
Liver abscess, %	3.2	2.2	4.3	5.4	5.9	5.4	2.31	0.96	0.43	0.90

<sup>1</sup>Treatment: C = Control and Z = zilpaterol-hydrochloride fed cattle

<sup>2</sup>P-values: Z = zilpaterol-hydrochloride; W = withdrawal time; Z×W = interaction

<sup>3</sup>Marbling scores were determined by USDA graders at a commercial abattoir; Small=400-499.

**Table A-4 Effects of zilpaterol-hydrochloride, withdrawal time, and wet aging on tenderness**

Withdrawal Day	Treatment <sup>1</sup>						SEM	P-Values <sup>2</sup>					
	C	Z	C	Z	C	Z		Z	W	A	Z×W	Z×A	Z×W×A
Wet aging, d													
7, kg	3.69	4.33	3.64	4.40	3.72	4.44	0.164	<0.001	0.311	<0.001	0.524	0.054	0.89
14, kg	3.42	3.81	3.18	3.66	3.18	3.87	0.164	<0.001	0.311	<0.001	0.524	0.054	0.89
21, kg	3.00	3.31	2.80	3.27	2.85	3.23	0.164	<0.001	0.311	<0.001	0.524	0.054	0.89

<sup>1</sup>Treatment: C = Control and Z = zilpaterol-hydrochloride

<sup>2</sup>P-values: Z = zilpaterol-hydrochloride; W = withdrawal time; A = aging time; Z×W = zilpaterol-hydrochloride by withdrawal interaction; and Z×W×A= zilpaterol-hydrochloride by withdrawal time by wet aging interaction

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