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Energy Status of Steers Dictates Effectiveness of Glycerol Inclusion in High-Roughage and High-Concentrate Feedlot Diets

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ENERGY STATUS OF STEERS DICTATES EFFECTIVENESS OF GLYCEROL INCLUSION IN HIGH-ROUGHAGE AND HIGH-CONCENTRATE FEEDLOT DIETS

BY

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This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science in Animal Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
BCS	Body condition score
BW	Body weight
CP	Crude protein
Cr-EDTA	Chromium ethylenediaminetetraacetate
DCAD	Dietary cation-anion difference
DHAP	Dihydroxyacetone phosphate
DIP	Degradable intake protein
DM	Dry matter
DMI	Dry matter intake
F:G	Feed:gain
G:F	Gain:feed
GLM	Generalized linear model
GLS	Glucose
GLYC	Glycerol
GRAS	Generally recognized as safe
HCW	Hot carcass weight
KPH	Kidney, pelvic, and heart fat
LMA	Longissimus muscle area
MP	Metabolizable protein
MUFA	Monounsaturated fatty acid

NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acid
NE_G	Net energy for gain
NE_M	Net energy for maintenance
OM	Organic matter
PUFA	Polyunsaturated fatty acid
PUN	Plasma urea nitrogen
SAS	Statistical Analysis System
SEM	Standard error of the mean
SFA	Saturated fatty acid
TMR	Total mixed ration
VFA	Volatile fatty acid
YG	Yield grade

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ABSTRACT

ENERGY STATUS OF STEERS DICTATES EFFECTIVENESS OF GLYCEROL INCLUSION IN HIGH-ROUGHAGE AND HIGH-CONCENTRATE FEEDLOT DIETS

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The objective of this research was to determine how to use glycerol as an effective source of energy in ruminant diets. Steer calves were used in a 56 d backgrounding study (n=128; Initial BW=340 ± 15 kg) and 105 d finishing study (n=120; Initial BW=420 ± 20 kg). Dietary treatments during backgrounding included 0, 8, 16, and 24% glycerol replacing corn silage in corn silage-based diets. Steers continued on within relative levels of dietary glycerol with finishing diets consisting of 0, 5, 10, and 15% glycerol replacing corn. Cumulative DMI, ADG, and G:F increased linearly (P<0.05) during backgrounding and decreased linearly (P<0.05) during finishing. The long-term feeding of glycerol did not affect carcass traits. In finishing steers, plasma glucose concentrations decreased linearly (P<0.05) in response to level of dietary glycerol. Plasma glycerol concentrations responded quadratically, with 15% glycerol diets resulting in lower plasma glycerol levels (P<0.05) than was found in Control steers. Changes in plasma glucose and glycerol concentrations suggest that hepatic function was impacted when glycerol was added to high grain content diets. Ruminal conditions associated with diets containing glycerol were characterized in ruminally fistulated steers fed low-quality forage or high-concentrate diets. Ruminal pH and concentrations of NH_3-N and VFA were within normal ranges. Regardless of diet, glycerol fermented to propionate and absorbed through the rumen epithelium accounted for 90% of ruminal losses. Ruminal escape of glycerol was estimated as 10% of

glycerol intake. Newly weaned and shipped steer calves (n=216; 287 ± 26 kg) were used in a 53 d feedlot receiving study. Dietary treatments consisted of 0, 8, 16, and 24% glycerol replacing dry rolled corn in sorghum silage-based diets. Dietary glycerol level did not affect DMI. From d 1 to 11, BW change increased linearly with increasing glycerol inclusion ($P<0.05$). Steers fed lower levels of glycerol exhibited linear, compensatory growth during the 12 to 22 d interim period ($P<0.05$). Plasma NEFA, glycerol, and glucose concentrations decreased linearly ($P<0.05$) and PUN concentrations increased linearly ($P<0.05$) in response to increasing dietary glycerol. Reduced DMI associated with high-concentrate diets is likely a physiological response due to the overabundance of glucose precursors relative to demand. Dietary glycerol hastened re-establishment of BW and normal glucose status in stressed calves.

KEYWORDS: cattle, concentrate, energy, glucose, glycerol, roughage

CHAPTER ONE:

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION

Considering feed costs contribute to approximately 70% of production expenses, beef producers are in constant search of cost-effective, alternative feedstuffs of appropriate nutrient composition. A by-product that has recently increased in availability and gained interest as a potential feedstuff is glycerol (or glycerin, glycerine). With increasing interest in the utilization of renewable fuel sources, the developing biodiesel industry has made the by-product, crude glycerol, more readily available as an economical feedstuff. Refined glycerin is used to manufacture numerous products including cosmetics, toiletries, pharmaceuticals and foodstuffs, and has industrial applications (Myers, 2007).

Glycerol has an energy value similar to corn (NE_M 2.20 Mcal/kg, NE_G 1.50 Mcal/kg; Preston, 2014). It contains no fat or protein and has minimal mineral content. A distinctive characteristic of glycerol is its ability to serve as a precursor for *de novo* glucose synthesis. Fisher et al. (1973) included glycerol in dairy cow diets as a means of treating ketosis, but glycerol use as a feed ingredient was limited by cost and availability. Parsons et al. (2009) more recently noted a depression in intake when glycerol was included up to 16% in finishing heifer diets and recommended effective inclusion levels to $\leq$ 2% of the diet. In contrast, Ramos and Kerley (2012) included glycerol up to 20% in forage-based diets replacing grass hay and observed no adverse effects on intake of heifers. The mechanism(s) behind these differences in the feeding value of glycerol when included in high-roughage and high-concentrate bovine diets are not well defined. The objective of this research was to further investigate whether the influence of glycerol on intake when included in high-roughage and high-concentrate diets is a physiological response relative to glucose status in feedlot steers.

LITERATURE REVIEW

Glycerol Production

The production of the renewable fuel source, biodiesel, generates crude glycerol as a co-product. Fat or oil is combined with an alcohol in the presence of a catalyst to produce crude biodiesel and glycerin through the process of transesterification. Soybean oil, methanol, and sodium hydroxide are most commonly used for biodiesel production in the United States. The fatty acids comprising triglycerides form an ester linkage with an alcohol during transesterification, yielding fatty acid esters and a three-carbon glycerol molecule. The mass difference between glycerol and biodiesel (methyl ester) allows for separation through a gravity process (Wisner, 2009). Biodiesel production generates approximately 10% glycerol (w/w) as the primary by-product (Yang et al., 2012). The world biodiesel market is expected to reach 37 billion gallons by 2016, which would result in the production of approximately 4 billion gallons of glycerol (Anand and Saxena, 2012).

Glycerin must undergo purification processes in order to be incorporated in food or drug products. Crude glycerin contains contaminants such as residual methanol, soaps, free fatty acids, spent catalysts, oils, and methyl esters. Glycerol is considered a generally recognized as safe (GRAS) animal feed ingredient. The U.S. Food and Drug Administration (FDA) considers crude glycerin derived from biodiesel with a methanol content above 150 ppm as unsafe for animal consumption (Sellers, 2008). Impurities such as residual sodium and potassium can affect electrolyte concentrations and dietary cation-anion difference (DCAD) when crude glycerin is used as a feedstuff (Yang et al., 2012).

Properties of Glycerol

Glycerol is a viscous, colorless, odorless liquid that has a relatively high boiling point (290°C), making it difficult to separate from other non-volatile impurities. Illustration 1. depicts the two-dimensional, chemical structure of glycerol. The three hydroxyl groups allow for hydrogen bonding which gives glycerol a viscous form and strong water holding capacity (Myers, 2007). Glycerol is completely soluble in water and alcohols and partially soluble in dioxane, ethyl acetate, and ether. The water binding property of glycerol makes it a suitable moisturizing agent. Purified glycerin is a valuable product used to manufacture pharmaceuticals, consumables, and cosmetics (Yang et al., 2012). At ambient temperature, refined glycerol exists in the liquid state and has a dry matter content of approximately 90% (Newman, 1968). The distinct physical and chemical characteristics of glycerol make it an exceptional feed additive in pelleting and increases flowability of liquid feeds. When feeding glycerol in extremely cold temperatures, the addition of up to 50% water can decrease the viscosity of the solution without freezing (Anderson and Ilse, 2008).

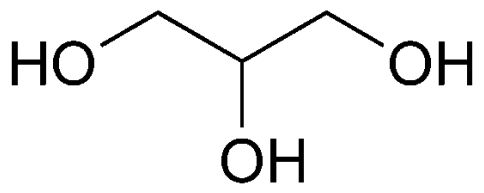


Illustration 1. Chemical structure of glycerol.

Glycerol as an Energy Source

The caloric value of glycerol is relatively equal to that of corn (NE_M=2.20 Mcal/kg; NE_G=1.50 Mcal/kg), but it contains no fat, protein, or fiber (Preston, 2014). Glycerol has been a feed additive of interest within the dairy industry as a means to enhance energy balance of cows. Ogborn (2006) administered a 500 mL oral bolus of glycerin to dairy cows 5 d postpartum and observed a decrease in plasma non-esterified fatty acid (NEFA) concentrations, suggesting the glucogenic property of dietary glycerol improved energy balance. Dietary glycerol had no influence on feed intake or milk production of cows fed 11.5% and 10.8% glycerol in prepartum and postpartum diets, respectively (Carvalho et al., 2011). During periods of heat stress, Liu et al. (2014) determined glycerol supplementation can enhance energy status and improve lactation performance of dairy cows. Dry glycerin (minimal 65% of food grade glycerol; dry powder) is an additional substrate that has been reported to have positive effects on the energy balance of dairy cows when top dressed during early lactation (Chung et al., 2007).

Glycerol has been supplemented during the feedlot receiving phase with the objective to increase energy intake and enhance immune function of feeder calves (Hales et al., 2013b). It is noteworthy that calves were offered long-stem wheat hay for up to 5 d post-arrival before treatments were initiated in this study. A linear decrease in DMI was observed when glycerol was included at levels up to 10% in receiving diets (25% roughage diet; control) replacing grass hay. Hales et al. (2013b) partially attributed the reduction in intake to an increase in energy density of the diet. Dietary glycerol was reported to have no influence on the number of calves treated for bovine respiratory disease, steers testing seropositive for bovine rhinotracheitis, or mortality rates. Although replacement of

roughage with glycerol did not alter the health status of high risk calves, Hales et al. (2013b) noted that glycerin improved feed efficiency and served as a viable feed ingredient when included as 5% of receiving diets.

Glycerol has been substituted for forage and grain in high-roughage bovine diets. Hales et al. (2013a) included glycerol at 7.5% of growing steer diets (40% roughage diet; control) replacing alfalfa or steam-flaked corn. There were no differences in intake and growth rate increased when glycerol replaced alfalfa (Hales et al., 2013a). Ramos and Kerley (2012) substituted grass hay with crude glycerin up to 20% of forage-based diets and reported no adverse impact on performance of heifers. Moriel et al. (2011) observed no influence on reproductive performance in developing replacement heifers fed 15% glycerol in diets based on bromegrass. The addition of glycerol in place of high-moisture corn in the total mixed ration (TMR) of transition dairy cows has been shown to increase feed consumption late in the day and reduce feed sorting (Carvalho et al., 2012).

The inclusion of glycerol in high-concentrate bovine diets has been reported to have variable effects on cattle performance. Parsons et al. (2009) observed a depression in intake when glycerol was included in finishing diets fed to heifers which limited effective inclusion levels to $\leq$ 2% of the diet. These results are consistent with those of Hales et al. (2015) as glycerol replaced dry rolled corn in finishing steer diets. In this finishing study, N retained (g/d) decreased while total heat production increased, suggesting the inclusion of glycerol in concentrate diets has a high metabolic cost (Hales et al., 2015). Anderson and Ilse (2008) noted including crude glycerin up to 18% in finishing diets substituting for dry-rolled corn and co-products resulted in a linear decrease in DMI but had no impact on gains of heifers. Mach et al. (2009) reported glycerol to have no detrimental effects on

performance when included up to 12% of isocaloric and isonitrogenous high-concentrate diets fed to Holstein bulls. Steers deprived of feed and water for 12 h and dosed with glycerol (2 g/kg BW) immediately prior to transportation maintained more body water compared to Control steers (Parker et al., 2007). Furthermore, steers dosed with glycerol had elevated blood glucose and insulin levels after 24 and 48 h of transportation, potentially inhibiting breakdown of muscle proteins and preserving muscle quality (Parker et al., 2007).

As a glucogenic compound, glycerol has been incorporated into finishing diets with the objective of improving carcass traits. In the finishing experiment performed by Mach et al. (2009), carcass and meat quality of Holstein bulls were not affected by dietary glycerol. Gunn et al. (2010) also demonstrated that crude glycerin can be added up to 15% in finishing diets of wethers without influencing carcass characteristics. In contrast, Parsons et al. (2009) reported a linear decrease in Longissimus muscle area, marbling, and subcutaneous fat in finishing heifers. This may have been due to the observed depression in intake associated with increasing glycerol inclusion. Krueger et al. (2010) proposed that dietary glycerol would enhance passage of unsaturated fatty acids from the rumen and improve small intestine absorption, making MUFA and PUFA more readily available for incorporation in meat. However, Avila-Stagno et al. (2013) observed no differences in total SFA or MUFA proportions of subcutaneous fat in lambs fed up to 21% dietary glycerol replacing dry rolled barley.

Rumen Parameters

Three of the principle fates of dietary glycerol in ruminants have been estimated and include absorption through the rumen epithelium (43%), fermentation to volatile fatty acids (44%), and passage to the small intestine (13%; Krehbiel, 2008). Werner Omazic et al. (2015) reported that approximately 45% of glycerol intake was absorbed from the rumen in non-lactating cows. Glycerol absorption appeared to occur primarily by passive diffusion rather than facilitated diffusion. Aquaporins are transport proteins that carry water and glycerol across cell membranes in various mammalian tissues including the rumen epithelium and gastrointestinal tract (Ishibashi et al., 2009). However, in this experiment performed by Werner Omazic et al. (2015), ruminal transport of glycerol was not impeded by the presence of an aquaporin inhibitor. Furthermore, transfer of glycerol across the rumen epithelium increased linearly with elevated glycerol levels, suggesting reliance upon carriers for absorption was minimal (Werner Omazic et al., 2015).

The bacterial species *Megasphaera elsdenii, Streptococcus bovis,* and *Selenomonas ruminantium* are primarily responsible for the anaerobic fermentation of glycerol. Lactic acid produced from the fermentation of glycerol is converted to butyrate by *M. elsdenii* (Stewart et al., 1997). A major source of propionate in the rumen is derived from the decarboxylation of succinate by *S. ruminantium* (Wolin et al., 1997). Using *in vitro* techniques, Hobson and Mann (1961) reported propionate to be the primary product of glycerol fermentation by these *selenomonads* in sheep ruminal fluid.

The inclusion of dietary glycerol in ruminant diets is noted to cause a shift in volatile fatty acid (VFA) profiles, favoring propionate production at the expense of acetate (Boyd et al., 2013). According to the computations of Rémond et al. (1993), approximately

35-69% of the carbons forming propionate originated from glycerol when ruminally dosed (240 g) in cows fed maize silage. Total VFA concentration has been reported to be unchanged (Hales et al., 2013) or to increase due to accumulation of propionate in steers fed glycerol (Wang et al., 2009).

Propionate is a glucogenic compound that conserves carbons and serves as a hydrogen sink. The ratio of acetate to propionate is elevated with increasing roughage in the diet. Considering glycerol is converted extensively to propionate in the rumen, Avila-Stagno et al. (2013) included increasing levels of glycerol in forage-based diets and evaluated CH_4 production using *in vitro* techniques. A linear increase in CH_4 emissions was observed with increasing glycerol inclusion which was attributed to the reduced state of glycerol in comparison to carbohydrates. The conversion of glycerol to propionate lacks the incorporation of net electrons; therefore, fermentation of glycerol to propionate in forage-based diets did not act as a hydrogen sink (Avila-Stagno et al., 2013).

Previous studies indicate that dietary glycerol lowers rumen ammonia (NH_3-N) concentrations. DeFrain et al. (2004) demonstrated this in dairy cows, suggesting increased utilization of NH_3-N by ruminal microbes for fermentation and growth. A linear reduction in rumen NH_3-N with increasing crude glycerin levels in corn stover-based diets was also reported by Wang et al. (2009). Considering cellulolytic bacteria obtain nitrogen exclusively from NH_3-N (Russell et al., 1992), there could have potentially been an increase in NH_3-N consumption associated with enhanced growth of cellulolytic bacteria populations.

Based on ruminal effective degradability of CP in steers fed high-concentrate diets, Wang et al. (2009) stated that proteolytic activity was impeded by glycerol

supplementation. Paggi et al. (1999) observed a 20% reduction in proteolytic activity when treating bovine rumen fluid with increasing levels of glycerol using *in vitro* techniques. Additionally, glycerol was estimated to inhibit *in vitro* ruminal lipolysis by nearly 48-77% (Krueger et al., 2010) and 46-80% (Edwards et al., 2012) without impacting rumen DM digestion.

Feed grade glycerol has been reported to decrease rumen pH levels without adversely affecting cellulolytic bacteria activity (Rémond et al., 1993). This decline in pH without detrimentally impacting cellulose digestion was also observed by Schröder and Südekum (1999) and Wang et al. (2009). In contrast, Roger et al. (1992) observed a decrease in cellulose degradation by cellulolytic bacteria *in vitro* with media containing 5% glycerol.

Glycerol Metabolism

The density of glycerol is 1.26 g/mL which is similar to the optimal density of particles passing from the rumen (Neel et al., 1995). Garton et al. (1961) evaluated the *in vitro* fermentation of glycerol using sheep rumen contents and reported that nearly 25% of the glycerol was undetectable by 2 h of incubation and over 90% of the glycerol had disappeared after 8 h of fermentation. Bergner et al. (1995) performed similar *in vitro* techniques and observed a glycerol disappearance rate of 90% within 2 h. Ruminal metabolism of glycerol was estimated to be 80% complete after 24 h of incubation *in vitro*, according to Trabue et al. (2007). Glycerol pulse dosed (480 g/d) in the rumen of cows fed maize silage was undetectable after 4 h (Remond et al., 1993).

Dietary glycerol has been reported to have negligible impact on nutrient

digestibility in ruminant diets. Boyd et al. (2013) observed no change in nutrient intake or apparent digestibility when glycerol was included up to 400 g/d in postpartum dairy cow diets. Similar results were observed in an experiment performed by Schróder and Südekum (1999) which involved feeding up to 20% glycerol in high-roughage diets for sheep. Winterholler et al. (2011) supplemented 860 g/d glycerol to beef cows during late gestation as a means to maintain body condition score (BCS) and observed no negative effects on total tract fiber digestibility. The use of glycerol as a replacement for roughage in growing steer diets resulted in a linear increase of apparent OM and apparent and true starch digestibility, while true OM digestibility responded quadratically (Hales et al., 2013). Nutrient digestibility of DM, OM, CP, NDF, and ADF increased linearly in the total tract of steers fed up to 300 g/d glycerol in corn stover-based diets (Wang et al., 2009).

Glycerol is rapidly absorbed from the gastrointestinal tract of young calves (Werner Omazic et al., 2013). This glucogenic compound is phosphorylated to glycerol-3-phsophate by glycerol kinase which is most active in hepatic tissue (Montell et al., 2002). Glycerol phosphate dehydrogenase oxidizes glycerol-3-phosphate to dihydroxyacetone phosphate (DHAP), generating NADH and H^+ (Montell et al., 2002). Depending on the energy status of the animal, DHAP enters either the glucogenic or glycolytic pathway.

When intake is low relative to maintenance, the demand for glucose is high and glycerol is directed towards *de novo* glucose synthesis. Since glycerol enters the pathway of gluconeogenesis at the triose phosphate level, it by-passes the rate-limiting enzymes pyruvate carboxylase and phosphoenolpyruvate carboxykinase (Krehbiel, 2008). During periods of stress or fasting, the body also mobilizes stored triglycerides as a source of energy, releasing glycerol and fatty acids into the bloodstream.

As intake increases above maintenance, the elevated supply of metabolites relative to demand results in storage of energy. Glycerol can enter glycolysis, by-passing the rate limiting enzymes hexokinase and phosphofructokinase, and be converted to acetyl-CoA for further metabolism via the TCA cycle (Allen et al., 2009). Glycerol is used to form triglycerides through an ester linkage with three fatty acids for storage in adipose tissue (Montell et al., 2002).

Blood metabolites of bovines supplemented with glycerol have been quantified to further evaluate the influence of this glucogenic compound on metabolism. When included in prepartum and postpartum diets at 11.5% and 10.8%, respectively, Carvalho et al. (2011) found blood glucose levels to be lower among cows fed glycerol. This response was proposed to be a depression in hepatic gluconeogenesis due to increased propionate production (Carvalho et al., 2011). Lactating dairy cows supplemented with glycerol during periods of heat stress had higher glucose and lower NEFA concentrations than control cows, suggesting dietary glycerol increased glucose utilization by peripheral tissues and lessened triglyceride mobilization (Liu et al., 2014). In an experiment performed by DeFrain et al. (2004), glucose and insulin levels were similar among transition dairy cows fed up to 7.2% dietary glycerol. These results are consistent with those of Mach et al. (2009) when crude glycerin was included up to 12% of isocaloric and isonitrogenous high-concentrate diets fed to Holstein bulls.

Summary

The developing biodiesel industry has expanded the availability of glycerol for use as a feed ingredient. The collection of literature evaluating glycerol as a feedstuff in ruminant diets suggest dietary glycerol has negligible impact on cellulolytic bacteria activity or nutrient digestibility (Wang et al., 2009; Winterholler et al., 2011). This glucogenic compound is a valuable feed additive in dairy diets as a means to enhance energy balance of cows (Liu et al., 2014). The influence of feed grade glycerol on intake when included in high-roughage and high-concentrate diets has created an interest in glycerol metabolism relative to glucose status in feedlot cattle.

CHAPTER TWO:

EVALUATION OF GLYCEROL INCLUSION IN BACKGROUNDING AND FINISHING STEER DIETS

INTRODUCTION

Utilization of alternative feedstuffs is one strategy that helps lower production expenses. With expansion of the biofuel industry, there has been an increase in the availability of glycerol. Glycerol has an energy value similar to corn, NE_M 2.20 Mcal/kg; NE_G 1.50 Mcal/kg (Preston, 2014). It contains no fat or protein and has minimal mineral content. The physical characteristics of glycerol make it an excellent diet conditioner. Glycerol is converted extensively to propionate in the rumen. A distinctive characteristic of glycerol is its ability to readily serve as a precursor for *de novo* glucose synthesis. There are mixed results on the usefulness of glycerol in ruminant diets. The potential of long-term feeding of glycerol to impact carcass characteristics has not been addressed. This experiment was designed to evaluate the effectiveness of glycerol as an energy source in backgrounding and finishing steer diets.

MATERIALS AND MEDTHODS

This experiment was conducted at the SDSU Ruminant Nutrition Center from November 2008 through May 2009. All experimental procedures used in this study were approved by the South Dakota State University Institutional Animal Care and Use Committee.

Animals and Treatments

Predominantly Angus steer calves were used in the 56 d backgrounding study (n=128; Initial BW=340 ± 15 kg) and 105 d finishing study (n=120; Initial BW=420 ± 20 kg). Backgrounding dietary treatments included 0, 8, 16, and 24% glycerol replacing corn silage in corn silage based diets (Table 2). Dietary treatments during the finishing phase consisted of 0, 5, 10, and 15% glycerol replacing corn in 9% roughage, finishing diets (Table 3). Prior to initiating the backgrounding experiment, steers were acclimated to feedlot conditions for 21 d. Steers were stratified by BW across 4 dietary treatments and then into 4 pen replicates within each treatment (7 or 8 steers/pen). Glycerol fed steers were adapted to the final backgrounding test diets within 7 d, transitioning from 8% glycerol to higher (16% and 24%) glycerol inclusion diets over 3 and 4 d, respectively.

Between the end of the backgrounding phase and onset of the finishing phase, steers were placed on a holding diet (12.8% CP; 1.25 Mcal/kg NE_G) based on high moisture ear corn for a 14 d period. During the transition period, 8 steers were removed from the population because of issues not related to treatment. Steers were re-allotted to pens within backgrounding treatment to normalize BW distributions for the finishing phase. Steers that were backgrounded on 0% glycerol diets remained on 0% glycerol diets in the finishing

phase. Steers previously fed 8% glycerol were assigned to the 5% glycerol finishing diet. Steers previously fed 16% glycerol were assigned to the 10% glycerol finishing diet, and steers previously fed 24% glycerol were assigned to the 15% glycerol finishing diet.

All diets were formulated to meet or exceed NRC nutrient requirements of growing steers (NRC, 1996). Backgrounding and finishing diets were formulated to provide 25 and 29 g/ton monensin, respectively. Diet ingredients were sampled weekly for nutrient analysis. Orts were removed, weighed, and sub-sampled prior to the morning feed delivery for determination of DM. Feed samples were dried in a forced air oven at 60°C for 24 h to determine DM content (AOAC, 1990) and ground through a 1mm screen. Ground feed samples were analyzed for NDF and ADF (Goering and Van Soest, 1970), CP (Kjeldahl procedure; AOAC, 1990), and ash content (AOAC, 1990). Actual diet formulations and compositions were calculated using weekly feed assay data and feed batching records.

Feed deliveries were made twice daily (0800 and 1400 h) in accordance with clean bunk management. Diets were mixed using a reel-type mixer and feed ingredients were added to the nearest 0.45 kg. On d 93 of the finishing study, high moisture ear corn was replaced with high moisture corn and additional oatlage to maintain similar diet composition. Feed records were summarized for each interim period corresponding to days that steers were weighed.

Individual BW were measured in the morning prior to feed delivery on d 21, 43, and 56 during backgrounding and d 33, 56, 84, and 105 of the finishing phase. Steers were implanted with Synovex-S (Zoetis; Kalamazoo, MI) on d 6 of backgrounding and 97 d later with Synovex-Choice (Zoetis; Kalamazoo, MI). This corresponded to d 33 of the finishing period. All interim steer performance data and cumulative live performance during

backgrounding were calculated without applying shrink. Cumulative live performance for the finishing study is reported using a 3% shrink as well as by using carcass adjusted final BW (HCW/0.625) to account for differences in fill.

Carcass Data Collection

Steers were marketed when the majority of the population was estimated to average 1.27 cm in ribfat thickness based on visual appraisal. Steers were co-mingled at 1600 h on d 105 of the finishing study and shipped as a single lot to Tyson Fresh Meats in Dakota City, NE. Individual steer identity was maintained throughout the packing plant and matched to camera grading data acquired from the abattoir.

Blood Sampling and Analysis

Four steers per pen were randomly selected for blood collection. Samples were acquired 4 h following the first feed delivery on d 85 of the finishing study. Blood was collected via jugular venipuncture using 18 gauge needles and 10 mL tri-potassium ethylenediaminetetraacetic acid vacuum-sealed tubes. Blood was centrifuged at 2,000 × g for 20 min at 4°C. Plasma was sorted into aliquots and stored in borosilicate glass tubes (13 × 100 mm) at -20°C.

Plasma samples were prepared for analyses by thawing and centrifuging at 430 × g for 10 min at 4°C to separate any coagulated plasma. Plasma glucose, glycerol, and urea nitrogen (PUN) concentrations were quantified using colorimetric spectrophotometry. Glucose (Autokit Glucose; Wako Diagnostics, Mountain View, CA) and glycerol (Free Glycerol Determination Kit; Sigma Aldrich, Saint Louis, MO) concentrations were

measured as hydrogen peroxide. The PUN assay used was based on the procedures described by Fawcett and Scott (1960). All samples were run in triplicate, allowing 5% variation between the high and low replicates.

Statistical Analysis

Treatment and pen replicate were included in the randomized complete block model (GLM, SAS; SAS Inst. Inc., Cary, NC) as independent sources of variation to evaluate the effect of dietary glycerol on steer performance using pen as the experimental unit. The model for evaluating the influence of dietary glycerol inclusion on carcass traits and blood metabolites included the same independent variables with individual steer as the experimental unit. Differences between means were considered significant at $P \leq 0.05$, and tendencies were reported at $0.05 \leq P \leq 0.10$. Contrast statements were used to determine if relationships between dietary treatment and the dependent variables were linear or quadratic.

RESULTS AND DISCUSSION

Steer Performance - Backgrounding

During the initial 21 d period, growth rate and G:F increased linearly ($P<0.05$) while DMI tended to increase linearly ($P<0.10$) as glycerol replaced corn silage in the backgrounding study (Table 4). From d 22 to 43, dietary glycerol treatment caused a linear increase in intake ($P<0.05$). From d 44 to 56 of the backgrounding study, ADG, DMI, and G:F increased linearly ($P<0.05$) with increasing glycerol inclusion. Cumulatively, increasing levels of dietary glycerol caused a linear increase in ADG, DMI, and G:F ($P<0.05$). Considering glycerol replaced a mixture of grain and roughage as silage, it is unclear whether the favorable responses in intake and gain were a positive associative effect of the glycerol or due to a reduction in NDF content of the diet. Hales et al. (2013a) included glycerol at 7.5% of growing steer diets (40% roughage diet; control) replacing alfalfa or steam-flaked corn. There were no differences in intake and growth rate increased when glycerol replaced alfalfa (Hales et al., 2013a).

Backgrounding diet NE_M and NE_G were derived from actual performance data (pen basis) using NE calculations published by Galyean (2005). When diet NE_M were regressed against dietary glycerol inclusion, the resulting equation was: $NE_M = 1.75 + 0.00753$ (% glycerol inclusion) ($P=0.04$; $r^2 =0.93$). When diet NE_G were regressed against dietary glycerol inclusion, the resulting equation was: $NE_G = 1.12 + 0.00675$ (% glycerol inclusion) ($P=0.04$; $r^2 =0.93$). Assuming the NE_M and NE_G values of corn silage are 1.65 Mcal/kg and 1.01 Mcal/kg, glycerol was calculated to have NE_M and NE_G values of 2.40 Mcal/kg and 1.68 Mcal/kg, respectively.

Steer Performance - Finishing

Glycerol inclusion did not affect steer performance ($P>0.10$) during the first 33 d of the finishing study which included the transition to high corn content finishing diets (Table 5). From d 34 to 56, intake decreased linearly ($P<0.05$) with increasing dietary glycerol. During d 57 to 84, dietary glycerol caused a linear decrease in ADG and DMI ($P<0.05$) while tending to decrease G:F ($P<0.10$). The final interim period of the finishing study (d 85 to 105) resulted in a linear decrease in ADG, DMI, and G:F ($P<0.05$) due to dietary treatment. Cumulatively, inclusion of up to 15% glycerol in finishing diets caused 15% lower ADG, 8% lower DMI, and no difference in G:F (Table 6). Cumulative carcass adjusted ADG decreased linearly ($P<0.05$) as glycerol increased in the diet. Although the inclusion of glycerol in high-concentrate diets depressed intake, cumulative absolute glycerol intake (kg/d) increased linearly ($P<0.05$). It is noteworthy that the absolute glycerol intake was higher for steers during backgrounding than in the finishing phase (Table 4 and 6). This suggests the depressed intake observed in high-concentrate diets was not an anti-nutritional characteristic of glycerol.

Parsons et al. (2008) noted a depression in intake when glycerol was included up to 16% in finishing diets replacing steam-flaked corn fed to heifers which limited effective inclusion levels to ≤ 2% of the diet. Hales et al. (2015) also observed a linear decrease in DMI for steers fed up to 15% glycerol in finishing diets. Comparison of current and previous research indicate that the acceptable inclusion level of glycerol in high concentrate diets may be limited. However, the dietary concentration of glycerol and total daily intake of glycerol can be much higher in high-roughage diets.

Finishing diet NE_M and NE_G were derived from actual performance data (pen basis)

using NE calculations published by Galyean (2005). When diet NE_M were regressed against dietary glycerol inclusion, the resulting equation was: NE_M = 2.12 - 0.0008 (% glycerol inclusion) (P=0.80; r^2 =0.04). When diet NE_G were regressed against dietary glycerol inclusion, the resulting equation was: NE_G = 1.45 - 0.0008 (% glycerol inclusion) (P=0.80; r^2 =0.04). The energy value of glycerol is apparently similar to corn. The overestimated NE_M and NE_G values of glycerol based on backgrounding performance data were potentially a positive associative effect of the glycerol in corn silage based diets.

Carcass Characteristics

Carcass Yield Grade data confirm that these cattle were fed to a common fat endpoint (Table 7). There were linear trends toward higher dressing percentage and lower HCW (P<0.10) with increasing glycerol inclusion. The higher dressing percentage would be consistent with lower DMI (i.e. less fill). Longissimus muscle area, rib fat depth, KPH, and marbling were not affected by treatment (P>0.10). Dietary glycerol had no impact on Quality Grade or Yield Grade. The abundance of glucogenic compounds associated with long-term feeding of glycerol did not improve intramuscular fat deposition. At the same time, depressed DMI caused by increasing glycerol inclusion in finishing diets did not reduce marbling. These results are supported by Mach et al. (2009) who observed no differences in carcass or meat quality of Holstein bulls fed up to 12% glycerol in high-concentrate diets. Gunn et al. (2010) also demonstrated that glycerol can be added up to 15% in finishing diets without influencing carcass characteristics of wethers. In contrast, Parsons et al. (2009) observed a linear decrease in LMA, marbling, and subcutaneous fat in finishing heifers fed up to 16% glycerol. It is unclear whether the differences in ribfat

depth of those heifers were due to reduced DMI associated with dietary glycerol. Considering the backgrounding allotment was carried over to the finishing phase in our experiment, glycerol fed steers may have put on more fat during backgrounding, allowing all steers to finish at a common fat endpoint.

Plasma Metabolites

The plasma glucose concentrations of finishing steers decreased linearly ($P<0.05$) in response to increasing dietary glycerol (Table 8). Plasma glycerol concentrations responded quadratically, increasing from 0 to 10% dietary glycerol with 15% glycerol diets resulting in plasma glycerol concentrations lower ($P<0.05$) than Control steers. There were no differences in PUN levels ($P>0.10$) in finishing steers fed increasing levels of glycerol. Whether glycerol is fermented to propionate or absorbed intact, both compounds can serve as precursors for *de novo* glucose synthesis. The fate of these glucogenic compounds depends on the energy status of the animal. The observed changes in blood glucose and glycerol concentrations in finishing cattle suggest that dietary glycerol altered hepatic metabolism which may be the source of a satiety signal causing lower DMI. Carvalho et al. (2011) reported a similar reduction in blood glucose (56.5 mg/dL, control; 52.5 mg/dL, glycerol) but found no differences in blood glycerol (0.75 mg/dL control; 0.74 mg/dL, glycerol) concentrations among dairy cows fed 11.5% and 10.8% glycerol in prepartum and postpartum diets, respectively. However, Liu et al. (2014) noted elevated plasma glucose levels and no differences in intake when glycerol was supplemented to lactating dairy cows during periods of heat stress. The similar PUN levels observed across treatments suggest dietary glycerol played a minor role in N metabolism of finishing steers.

IMPLICATIONS

When backgrounding calves on corn silage, dietary glycerol increased feed intake and growth rate. Actual performance data confirm that glycerol has an energy value similar to corn. Reduced DMI associated with glycerol added to high-concentrate diets was apparently not an anti-nutritional characteristic of glycerol, since the absolute glycerol intake (kg/d) was higher for steers backgrounded on corn silage with glycerol. The long-term feeding of glycerol did not influence Yield Grade or Quality Grade. The effectiveness of glycerol inclusion in high starch diets may be limited due to the abundance of glucogenic compounds relative to demand in finishing steers.

Table 1. Backgrounding study formulas for pelleted supplement batches.[1]

	Glycerol inclusion, %			
	0	**8**	**16**	**24**
Ingredient	**Kilograms**			
Soybean meal	792	797	798	799
Limestone	84	76	69	64
Trace mineralized salt	29	25	22	20
Potassium chloride	--	7	16	22
Microingredients[2]	2	2	2	2

[1] As is basis.
[2] Microingredients included monensin, vitamins A & E, $ZnSO_4$, and $CuSO_4$.

Table 2. Backgrounding diet formulations and compositions.[1]

	Glycerol inclusion, %			
	0	**8**	**16**	**24**
1-3 d				
Corn silage, %	82.41	72.97	72.97	72.97
Dried distillers grains, %	8.51	8.55	8.55	8.55
Glycerol, %	--	7.66	7.66	7.66
Pelleted supplement, %[2]	9.08	10.82	10.82	10.82
4-7 d				
Corn silage, %	82.41	72.97	63.49	63.49
Dried distillers grains, %	8.51	8.55	8.61	8.61
Glycerol, %	--	7.66	15.42	15.42
Pelleted supplement, %[2]	9.08	10.82	12.48	12.48
8-56 d				
Corn silage, %	83.13	74.15	64.80	55.26
Dried distillers grains, %	8.35	8.41	8.48	8.55
Glycerol, %	--	7.58	15.28	23.11
Pelleted supplement, %[2]	8.52	9.86	11.44	13.08
DM, %[3]	38.65	41.19	44.21	47.77
CP, %[3]	12.09	11.97	12.03	12.09
NDF, %[3]	41.63	36.99	34.39	28.49
NE_M, Mcal/kg[4]	1.73	1.80	1.86	1.93
NE_G, Mcal/kg[4]	1.10	1.15	1.20	1.26

[1] All values except DM on DM basis.
[2] Provides vitamins and minerals to meet or exceed nutrient requirements (NRC, 1996). Monensin included to provide 25 g/T. (Table 1).
[3] Based on weekly ingredient analyses.
[4] Based on tabular NE values of ingredients fed.

Table 3. Finishing diet formulations and compositions.[1]

	Glycerol inclusion, %			
	0	**5**	**10**	**15**
1-92 d				
Oatlage, %	3.89	3.89	3.89	3.89
High moisture ear corn, %	25.09	25.09	25.08	25.08
Whole shelled corn, %	58.39	52.09	45.80	39.53
Glycerol, %	--	5.01	10.01	15.01
Liquid supplement, %[2]	4.25	4.25	4.25	4.25
Pelleted supplement, %	8.39	9.68	10.97	12.24
Soybean meal[3]	(4.15)	(4.79)	(5.43)	(6.06)
Dried distillers grains[3]	(4.15)	(4.79)	(5.43)	(6.06)
Limestone[3]	(0.09)	(0.10)	(0.11)	(0.12)
DM, %[4]	77.61	77.81	78.01	78.20
CP, %[4]	12.64	12.67	12.69	12.72
NDF, %[4]	15.02	14.73	14.43	14.14
NE_M, Mcal/kg[5]	2.04	2.05	2.07	2.08
NE_G, Mcal/kg[5]	1.37	1.38	1.38	1.39
93-105 d				
Oatlage, %	8.75	8.75	8.75	8.75
High moisture corn, %	21.67	21.68	21.68	21.68
Whole shelled corn, %	57.16	51.08	45.04	38.97
Glycerol, %	--	4.89	9.78	14.67
Liquid supplement, %[2]	4.16	4.15	4.16	4.15
Pelleted supplement, %	8.26	9.45	10.59	11.78
Soybean meal[3]	(4.09)	(4.68)	(5.24)	(5.83)
Dried distillers grains[3]	(4.09)	(4.68)	(5.24)	(5.83)
Limestone[3]	(0.08)	(0.09)	(0.11)	(0.12)
DM, %[4]	77.21	77.38	77.56	77.73
CP, %[4]	12.90	12.90	12.89	12.89
NDF, %[4]	14.37	14.08	13.79	13.50
NE_M, Mcal/kg[5]	2.06	2.07	2.08	2.09
NE_G, Mcal/kg[5]	1.37	1.38	1.39	1.40

[1] All values except DM on DM basis.
[2] Provides vitamins and minerals to meet or exceed nutrient requirements (NRC, 1996). Monensin added to provide 29 g/T.
[3] Values in parentheses are totaled as pelleted supplement.
[4] Based on weekly ingredient analyses.
[5] Based on tabular NE values of ingredients fed.

Table 4. Steer performance responses to 0, 8, 16, and 24% glycerol in backgrounding diets.[1]

	Glycerol inclusion, %					Contrasts, *P*-value[2]	
	0	**8**	**16**	**24**	**SEM[3]**	**Linear**	**Quadratic**
Initial BW, kg	344	344	343	343	0.635	--	--
1-21 d							
d 21 BW, kg	376	377	380	381	1.8	0.04	--
ADG, kg	1.50	1.58	1.71	1.82	0.086	0.02	--
DMI, kg	7.48	7.63	7.67	7.71	0.082	0.08	--
G:F	0.201	0.207	0.223	0.237	0.010	0.02	--
F:G	4.99	4.88	4.52	4.25	0.205	0.02	--
22-43 d							
d 43 BW, kg	399	410	406	413	2.6	<0.01	--
ADG, kg	1.05	1.47	1.22	1.44	0.140	0.18	--
DMI, kg	8.21	9.18	9.08	9.17	0.230	0.02	0.09
G:F	0.127	0.160	0.134	0.156	0.013	--	--
F:G	8.07	6.37	7.47	6.71	0.667	--	--
44-56 d							
d 56 BW, kg	416	430	430	440	1.8	<0.01	--
ADG, kg	1.31	1.55	1.84	2.07	0.089	<0.01	--
DMI, kg	8.80	9.58	9.78	10.07	0.196	<0.01	--
G:F	0.149	0.162	0.189	0.205	0.011	<0.01	--
F:G	6.74	6.19	5.38	4.92	0.312	<0.01	--
Cumulative							
ADG, kg	1.28	1.53	1.55	1.73	0.040	<0.01	--
DMI, kg	8.07	8.69	8.71	8.83	0.129	<0.01	0.08
G:F	0.158	0.176	0.178	0.196	0.003	<0.01	--
F:G	6.32	5.68	5.63	5.11	0.083	<0.01	--
Glycerol intake, kg/d[4]							
	--	0.66	1.33	2.04	0.020	<0.01	--

[1] Non-shrunk BW basis.
[2] Probability > 0.20 not depicted.
[3] n=4 pens/treatment.
[4] Cumulative absolute glycerol intake (kg/d).

Table 5. Interim periods steer performance responses to 0, 5, 10, and 15% glycerol in finishing diets.[1]

	Glycerol inclusion, %					Contrasts, *P*-value[2]	
	0	**5**	**10**	**15**	**SEM[3]**	**Linear**	**Quadratic**
Initial BW, kg	413	421	421	426	3.4	0.03	--
1-33 d							
d 33 BW, kg	489	494	497	501	3.9	0.05	--
ADG, kg	2.28	2.22	2.29	2.26	0.073	--	--
DMI, kg	8.46	8.41	8.42	8.37	0.092	--	--
G:F	0.270	0.264	0.272	0.270	0.009	--	--
F:G	3.73	3.80	3.69	3.71	0.125	--	--
34-56 d							
d 56 BW, kg	538	544	543	545	4.7	--	--
ADG, kg	2.12	2.17	1.99	1.91	0.130	0.19	--
DMI, kg	10.95	10.93	10.29	10.19	0.252	0.03	--
G:F	0.193	0.199	0.193	0.186	0.010	--	--
F:G	5.20	5.14	5.21	5.41	0.271	--	--
57-84 d							
d 84 BW, kg	595	592	585	589	4.5	--	--
ADG, kg	2.06	1.72	1.53	1.59	0.079	<0.01	0.03
DMI, kg	11.54	11.13	10.38	10.28	0.242	<0.01	--
G:F	0.178	0.155	0.148	0.155	0.008	0.08	0.09
F:G	5.64	6.53	6.85	6.49	0.324	0.08	0.09
85-105 d							
d 105 BW, kg	635	627	618	614	4.7	<0.01	--
ADG, kg	1.92	1.64	1.56	1.19	0.064	<0.01	--
DMI, kg	12.02	11.55	10.63	10.38	0.255	<0.01	--
G:F	0.160	0.142	0.146	0.115	0.007	<0.01	--
F:G	6.29	7.08	6.88	8.94	0.473	<0.01	--

[1] Non-shrunk BW basis.
[2] Probability > 0.20 not depicted.
[3] n=4 pens/treatment.

Table 6. Cumulative steer performance responses to 0, 5, 10, and 15% glycerol in finishing diets.

	Glycerol inclusion, %					Contrasts, *P*-value[1]	
Item	0	5	10	15	SEM	Linear	Quadratic
Shrunk Basis[2]							
Final BW, kg	616	608	599	596	4.6	<0.01	--
ADG, kg	2.05	1.90	1.82	1.73	0.029	<0.01	--
DMI, kg	10.54	10.31	9.80	9.68	0.173	<0.01	--
G:F	0.195	0.184	0.186	0.179	0.003	0.01	--
F:G	5.15	5.42	5.38	5.58	0.086	0.01	--
Glycerol intake, kg/d[3]							
	--	0.52	0.98	1.46	0.020	<0.01	--
Carcass Adjusted Basis[4]							
Final BW, kg	619	611	607	604	5.0	0.06	--
ADG, kg	2.08	1.94	1.90	1.81	0.036	<0.01	--
G:F	0.197	0.187	0.193	0.188	0.003	0.17	--
F:G	5.08	5.34	5.17	5.34	0.090	0.17	--

[1] Probability > 0.20 not depicted.
[2] 3% shrink applied to initial BW and d 105 BW.
[3] Cumulative absolute glycerol intake (kg/d).
[4] Calculated Final BW= HCW/0.625.

Table 7. Influence of long-term feeding of glycerol on carcass traits.[1]

	Glycerol inclusion, %					Contrasts, *P*-value[2]	
Item	**0**	**5**	**10**	**15**	**SEM**	**Linear**	**Quadratic**
Dress, %[3]	62.77	62.84	63.31	63.37	0.252	0.08	
HCW, kg	387	382	380	377	3.1	0.06	--
LMA, cm^2	86.77	85.03	84.06	84.84	1.2	--	--
Ribfat, cm	1.47	1.52	1.60	1.47	0.069	--	0.19
KPH, %	1.93	2.00	2.00	1.98	0.029	--	0.12
Marbling[4]	573	583	583	579	20.1	--	
Yield Grade	3.25	3.35	3.45	3.28	0.120	--	--

[1] Individual carcass basis.
[2] Probability > 0.20 not depicted.
[3] HCW as % of shrunk BW.
[4] 400 = Slight°; 500 = Small°.

Table 8. Plasma metabolites of steers fed 0, 5, 10, and 15% glycerol in finishing diets.[1]

	Glycerol inclusion, %					Contrasts, *P*-value[2]	
Item	**0**	**5**	**10**	**15**	**SEM**	**Linear**	**Quadratic**
Glucose, mg/dL	79.54	73.10	70.92	71.48	2.3	0.03	0.16
Glycerol, mg/dL	1.88	2.93	3.79	1.23	0.200	--	<0.01
PUN, mg/dL	9.93	9.68	9.65	9.58	0.526	--	--

[1] Individual steer basis.

[2] Probability > 0.20 not depicted.

CHAPTER THREE:

ESTIMATING THE RELATIVE PROPORTIONS OF GLYCEROL FATES IN THE RUMEN OF STEERS FED HIGH-ROUGHAGE AND HIGH-CONCENTRATE DIETS

INTRODUCTION

The balance of rumen function and metabolite flow influences feed intake of ruminants. Consistent with previous research, we observed a depression in intake as glycerol replaced corn in finishing diets. In contrast, we observed an increase in DMI when glycerol was substituted for corn silage in backgrounding diets. Intake is regulated by fill in high-roughage diets, and the NDF content decreased as glycerol replaced corn silage. Reduced DMI associated with high-concentrate diets was apparently not an anti-nutritional effect of dietary glycerol, since the absolute glycerol intake (kg/d) was higher for steers backgrounded on corn silage.

The fates of glycerol in the rumen include fermentation to volatile fatty acids, absorption across the rumen epithelium, and outflow to the small intestine (Krehbiel, 2008). It is not well defined whether the relative proportions of these fates are different when glycerol is included in roughage and concentrate-based diets. Potential differences in rumen pH, metabolizable protein (MP) supply, or propionate production caused by dietary glycerol could shift the proportions of glycerol being fermented, absorbed, and reaching the small intestine. This experiment was designed to determine whether the influence of dietary glycerol on intake is a ruminal or physiological response when included in high-roughage and high-concentrate bovine diets.

MATERIALS AND METHODS

This experiment was conducted at the SDSU Ruminant Nutrition Center in November 2013 and August 2014. All experimental procedures used in this study were approved by the South Dakota State University Institutional Animal Care and Use Committee.

Animals and Diets

Exp. 1. Mature, Angus and SimAngus steers (n=5) were fed 15% glycerol in a low-quality forage diet (Table 9). Steers were fed once daily (0730 h) in accordance with slick bunk management. The diet was formulated to meet NRC nutrient requirements for maintenance of mature beef cows (NRC, 1996). Soybean hulls, supplement, and glycerol were mixed in a stationary ribbon mixer and delivered separately from the grass hay. The daily grass hay allotment was offered following consumption of the concentrate mix. Steers were adapted to the diet over a 21 d period prior to collection of rumen fluid samples.

Exp. 2. Growing, Limousin × Jersey steers (n=4) were fed 15% glycerol in a high-concentrate diet (Table 10). Steers were fed twice daily (0700 h and 1400 h) in accordance with slick bunk management. The diet was formulated to meet or exceed NRC nutrient requirements of finishing steers (NRC, 1996). The daily ration of dry rolled corn, dried distillers grains with solubles, supplement, and glycerol was mixed in a stationary ribbon mixer prior to the afternoon feeding. The roughage portion of the diet was added to the concentrate mix at the time of delivery. Three transition diets were used to allow for adaption to the final diet, and steers were fed to reach ad libitum intake. All steers used in this experiment were housed individually to monitor intake.

For each experiment, diet ingredients were sampled weekly for nutrient analysis. Orts were removed, weighed, and sub-sampled prior to the morning feed delivery for determination of DM. Feed samples were dried in a forced air oven at 60°C for 24 h to determine DM content (AOAC, 1990) and subsequently ground through a 1 mm screen. Ground feed samples were analyzed for NDF and ADF (Goering and Van Soest, 1970), CP (Kjeldahl procedure; AOAC, 1990), and ash content (AOAC, 1990). Actual diet formulations and compositions were calculated using weekly feed assay data and feed batching records.

Ruminal Fluid Collection and Analysis

Exp. 1. Mature steers fed the low-quality forage diet were provided half of the daily concentrate mix on d 22. Following consumption of the concentrate mix, the remaining half was ruminally dosed along with 1 L of Cr-EDTA (2770 ppm Cr) and the daily hay allotment was offered. Ruminal fluid was collected via rumen cannula at -1, 0.5, 3, 6, 9, 12, and 24 h from the time of dosing with Cr-EDTA and concentrate mix.

Exp. 2. Growing steers fed the high-concentrate diet were provided the morning feed delivery without glycerol on d 47. The glycerol that was withheld from the morning feed mix was ruminally dosed along with 300 mL of Cr-EDTA (831 ppm Cr) following feeding. The complete ration was delivered for the afternoon feeding. Ruminal fluid was collected via rumen cannula at -1, 0.5, 3, 6, 9, 12, and 24 h from the time of dosing with Cr-EDTA and glycerol.

Ruminal fluid samples accessed via the rumen fistula were immediately strained through four layers of cheesecloth and pH was recorded using a benchtop pH meter.

Aliquots (30 mL) for Cr and NH_3-N analysis were transferred directly into vials and frozen at -20°C. A 5 mL aliquot was acidified with 1 mL of 25% (wt/vol) meta-phosphoric acid and frozen at -20°C for subsequent glycerol and VFA analysis.

Ruminal fluid samples were prepared for analysis by thawing and centrifuging at 20,000 × *g* for 30 min at 4°C. The supernatant was collected and sorted into aliquots. The VFA concentrations were determined by gas chromatography using an 1829 mm × 6 mm column (Supelco, Bellefonte, PA) packed with 10% SP-1200 + 1% H_3PO_4 on 80/100 Chromosorb WAW. The column oven was operated at 140°C with a helium flow rate of 50 mL/min (Erwin et al., 1961). Rumen NH_3-N and glycerol concentrations were analyzed using colorimetric spectrophotometry. The NH_3-N assay used was a phenol-hypochlorite reaction based on the procedures of Weatherburn (1967). Glycerol was phosphorylated to glycerol-1-phosphate by glycerol kinase and then oxidized to dihydroxyacetone phosphate (DHAP) and hydrogen peroxide by glycerol phosphate oxidase (Free Glycerol Determination Kit; Sigma Aldrich, Saint Louis, MO). Chromium EDTA was prepared according to the procedures of Binnerts et al. (1968). Chromium concentrations were determined by atomic absorption using an air-acetylene flame with oxidant and acetylene flow rates of 10 L/min and 2.5 L/min, respectively (Binnerts et al., 1968). Samples for VFA analysis were run in duplicate and all other sample analyses were run in triplicate, allowing 5% variation between high and low replicates.

Ruminal Kinetics Calculations

Fluid passage rate and glycerol disappearance rate were calculated by regressing the natural log of Cr and glycerol concentration, respectively, on time after dosing (Warner

and Stacy, 1968). The regression equation used was: y = a + bx where y = ln [Cr] or ln [glycerol] and x = time. Ruminal fluid volume was estimated by dividing the dose concentration of Cr by the extrapolated Cr concentration of rumen contents at the time of dosing (Warner and Stacy, 1968). The ruminal disappearance rate of glycerol was compared to that of Cr to estimate the proportion of glycerol apparently fermented or absorbed through the rumen epithelium. Glycerol outflow (%) was estimated by calculating fluid passage rate as a percentage of the glycerol disappearance rate: (Fluid passage rate ÷ glycerol disappearance rate) × 100.

Statistical Analysis

This study was not designed to test treatments. Data were used for the relative estimation of glycerol fates and characterization of rumen parameters over time. Time and individual steer were included in the model (GLM, SAS; SAS Inst. Inc., Cary, NC) as independent sources of variation to analyze rumen pH, NH_3-N, and VFA concentrations in response to time post-dosing. Dependent variable changes over time were separated using Duncan's multiple range test. Chromium and glycerol concentrations and ruminal kinetics data were compiled using simple means by time.

RESULTS AND DISCUSSION

Rumen Parameters

Steers were consuming 12.2 kg of the low-quality forage diet and 9.98 kg of the high-concentrate diet at the time of rumen sample collections. Steers fed the high-concentrate diet experienced warm weather conditions (31°C) on the day of sample collection which altered their intake pattern and is partially reflected in the diurnal pattern of rumen parameters.

Ruminal pH and NH_3-N concentrations declined ($P<0.05$) following feeding and the ruminal glycerol dose. These variables returned to basal levels by 24 h in steers fed low-quality forage and high-concentrate diets (Table 11 and 12). Ruminal pH levels measured over time were characteristic of ruminants consuming roughage and concentrate diets (Rumsey et al., 1970). Rémond et al. (1993) demonstrated that dietary glycerol can cause a decrease in ruminal pH levels without adversely affecting cellulolytic bacteria activity. Wang et al. (2009) also reported a linear reduction in rumen NH_3-N concentrations with increasing glycerol inclusion in corn stover-based diets.

Both diets included a spike in propionate concentration following the ruminal glycerol dose which decreased ($P<0.05$) the acetate to propionate ratio over time. Butyrate, isovalerate, and valerate concentrations increased ($P<0.05$) post-dosing in steers fed the low-quality forage diet while acetate and isobutyrate were unchanged ($P>0.10$). Steers fed the high-concentrate diet had higher ($P<0.05$) butyrate and lower ($P<0.05$) acetate, isobutyrate, and isovalerate concentrations following the ruminal glycerol dose while valerate was unchanged ($P>0.10$). There were no observed increases in VFA concentrations following the afternoon feed delivery in steers fed the high-concentrate diet

which was likely due to warm weather conditions altering their intake patterns.

Total VFA included acetate, propionate, isobutyrate, butyrate, isovalerate, and valerate concentrations. Total VFA concentrations increased ($P<0.05$) over time in steers fed glycerol in the low-quality forage diet and were unchanged ($P>0.10$) in steers fed the high-concentrate diet. Boyd et al. (2013) reported a shift in the volatile fatty acid profiles of dairy cows fed glycerol, favoring propionate production at the expense of acetate (i.e. no change in total VFA concentrations). Wang et al. (2009) observed an increase in total VFA concentrations due to accumulation of propionate in steers fed glycerol in corn stover-based diets. Comparison of current and previous research indicate that dietary glycerol has no adverse impact on rumen parameters when included in high-roughage or high-concentrate bovine diets.

Ruminal kinetics

Calculated rumen fluid volumes (Table 13) were representative of mature cattle fed high-roughage diets (70-90 L) and growing cattle fed high-concentrate (40-60 L) diets (Church, 1988). Fluid passage rates were characteristic of steers consuming roughage (8-10 %/h) and concentrate-based (5-7 %/h) diets (Church, 1988).

Glycerol disappeared from the rumen at a faster rate in steers fed 15% glycerol in the low-quality forage diet. Bergner et al. (1995) observed a glycerol disappearance rate of 90% within 2 h *in vitro* using sheep ruminal fluid with wheat starch added as a substrate. Trabue et al. (2007) estimated ruminal metabolism of glycerol *in vitro* to be 80% complete after 24 h of incubation when glycerol was added to ruminal fluid from dairy cows. These disappearance rates reported by Bergner et al. (1995) and Trabue et al. (2007) are strictly

representative of glycerol fermentation and do not account for glycerol absorption or passage from the rumen.

The estimated rate of glycerol apparently fermented or absorbed intact through the rumen epithelium was greater for steers fed the roughage diet. In spite of differences in fluid passage rate and glycerol disappearance rate, estimated glycerol outflow was approximately 10% of glycerol intake in both diets. Werner Omazic et al. (2015) reported that approximately 45% of glycerol intake was absorbed from the rumen in non-lactating cows.

Since ruminal conditions appeared normal in these experiments, there must be some other factor triggering satiety when glycerol is added to high-concentrate diets. In the hepatic oxidation theory, feed intake is regulated by the oxidation of fuels at the liver (Allen et al., 2009). Considering finishing cattle can consume up to 2.9 × maintenance of high-starch diets, the concentrations of glucose precursors (i.e. propionate and glycerol) reaching the liver likely caused the triggering of a satiety signal resulting in depressed intake.

IMPLICATIONS

Even after the perturbed conditions of providing the adapted 24 h glycerol intake in a pulse dose, ruminal pH and NH_3-N and VFA concentrations were within normal ranges. Estimated glycerol outflow was approximately 10% of glycerol intake in steers fed either low-quality forage or high-concentrate-based diets. Dietary glycerol fermentation to propionate or glycerol absorption intact through the rumen epithelium accounted for 90% of glycerol consumed. Since rumen parameters were normal, depressed intake associated with glycerol inclusion in high-concentrate diets is likely a physiological response due to the overabundance of glucose precursors relative to demand.

Table 9. Formulation and composition of low-quality forage diet.[1]

Grass hay, %	65.63
Soybean hulls, %	15.64
Glycerol, %	14.85
Pelleted supplement, %[2]	3.88
Soybean meal[3]	(3.22)
Limestone[3]	(0.47)
Trace mineralized salt[3]	(0.17)
Microingredients[3,4]	(0.02)
DM, %[5]	90.23
CP, %[5]	7.77
NDF, %[5]	52.26
NE_M, Mcal/kg[6]	1.43
NE_G, Mcal/kg[6]	0.80

[1] All values except DM on DM basis.
[2] Provides vitamins and minerals to meet or exceed nutrient requirements (NRC, 1996).
[3] Values in parentheses are totaled as pelleted supplement.
[4] Microingredients include monensin: 255 g, vitamin A & E, $ZnSO_4$, and $CuSO_4$.
[5] Based on weekly ingredient analyses.
[6] Based on tabular NE values of ingredients fed.

Table 10. Formulation and composition of high-concentrate diet.[1]

Sorghum silage, %	13.11
Dry rolled corn, %	47.80
Dried distillers grains, %	20.47
Glycerol, %	15.26
Supplement, %[2]	3.36
Canola meal[3]	(0.52)
Limestone[3]	(1.75)
Potassium chloride[3]	(0.50)
Trace mineralized salt[3]	(0.30)
Urea[3]	(0.25)
Ground corn[3]	(0.02)
Microingredients[3,4]	(0.02)
DM, %[5]	65.15
CP, %[5]	12.47
NDF, %[5]	15.98
NE_M, Mcal/kg[6]	2.09
NE_G, Mcal/kg[6]	1.34

[1] All values except DM on DM basis.
[2] Provides vitamins and minerals to meet or exceed nutrient requirements (NRC, 1996).
[3] Values in parentheses are totaled as meal supplement.
[4] Microingredients include monensin: 838 g, vitamin A & E, $ZnSO_4$, and $CuSO_4$.
[5] Based on weekly ingredient analyses.
[6] Based on tabular NE values of ingredients fed.

Table 11. Rumen parameters over time in mature steers ruminally dosed with glycerol and fed a low-quality forage diet.

	Time, h[1]								*P*-value
	-1	**0.5**	**3**	**6**	**9**	**12**	**24**	**SEM[2]**	**Time**
pH	7.61^{ab}	6.95^{cd}	5.66^{e}	6.72^{d}	7.11^{c}	7.29^{bc}	7.89^{a}	0.125	<0.01
NH_3-N, mg/dL	4.01^{a}	3.32^{ab}	1.82^{cd}	1.56^{d}	1.15^{d}	1.13^{d}	2.79^{bc}	0.364	<0.01
VFA, mmol/L									
Acetate (A)	33.33	38.65	31.41	41.52	40.47	42.27	37.36	2.9	0.09
Propionate (P)	7.81^{a}	18.15^{cd}	21.14^{d}	30.58^{e}	18.31^{cd}	13.66^{bc}	9.09^{ab}	1.7	<0.01
Isobutyrate	0.36	0.34	0.25	0.31	0.20	0.27	0.49	0.072	0.17
Butyrate	2.64^{a}	6.87^{d}	5.61^{cd}	5.85^{cd}	4.37^{bc}	3.94^{ab}	2.98^{ab}	0.485	<0.01
Isovalerate	0.59^{abc}	0.72^{a}	0.56^{abc}	0.41^{c}	0.42^{c}	0.52^{bc}	0.64^{ab}	0.061	0.01
Valerate	0.28^{a}	0.69^{c}	0.67^{c}	0.76^{c}	0.59^{bc}	0.48^{b}	0.28^{a}	0.056	<0.01
Total VFA	45.01^{a}	65.41^{c}	59.64^{bc}	79.43^{d}	64.36^{bc}	61.15^{bc}	50.84^{ab}	4.5	<0.01
A:P	4.39^{a}	2.16^{c}	1.86^{cd}	1.38^{d}	2.30^{c}	3.16^{b}	4.24^{a}	0.224	<0.01

[1] Relative to Cr-EDTA and glycerol dose, 1 h following morning feed delivery.
[2] n=5 steers/sample time.
$^{a\,b\,c\,d\,e}$ Means within a row without a common superscript differ (P<0.05).

Table 12. Rumen parameters over time in growing steers ruminally dosed with glycerol and fed a high-concentrate diet.

	Time, h[1]								*P*-value[3]
	-1	**0.5**	**3**	**6**	**9**	**12**	**24**	**SEM[2]**	**Time**
pH	6.53[a]	5.83[c]	5.33[d]	6.01[bc]	6.52[a]	6.44[ab]	6.84[a]	0.146	<0.01
NH_3-N, mg/dL	11.37[ab]	11.78[a]	5.18[c]	8.86[b]	9.29[b]	6.42[c]	9.23[b]	0.785	<0.01
VFA, mmol/L									
Acetate (A)	56.04[a]	47.45[ab]	30.74[d]	34.52[cd]	41.47[bc]	45.19[b]	45.18[b]	3.1	<0.01
Propionate (P)	26.22[ab]	36.96[cd]	43.07[d]	52.78[e]	36.89[cd]	32.06[bc]	22.48[a]	2.8	<0.01
Isobutyrate	0.87[a]	0.80[a]	0.30[b]	0.24[b]	0.38[b]	0.41[b]	0.74[a]	0.061	<0.01
Butyrate	13.26[b]	18.65[a]	11.41[bc]	8.40[c]	9.98[bc]	11.54[bc]	10.26[bc]	1.2	<0.01
Isovalerate	2.59[a]	2.44[a]	1.29[b]	1.23[b]	1.35[b]	1.38[b]	2.32[a]	0.177	<0.01
Valerate	1.33	1.58	1.21	1.54	1.67	1.46	1.03	0.178	0.20
Total VFA	100.30	107.88	88.01	98.72	91.73	92.04	82.02	6.9	--
A:P	2.23[a]	1.30[bc]	0.71[d]	0.67[d]	1.12[c]	1.43[b]	2.09[a]	0.091	<0.01

[1] Relative to Cr-EDTA and glycerol dose, 1 h following morning feed delivery. Feed was delivered 7 h post-dosing in the afternoon.
[2] n=4 steers/sample time.
[3] Probability > 0.20 not depicted.
[a b c d e] Means within a row without a common superscript differ ($P<0.05$).

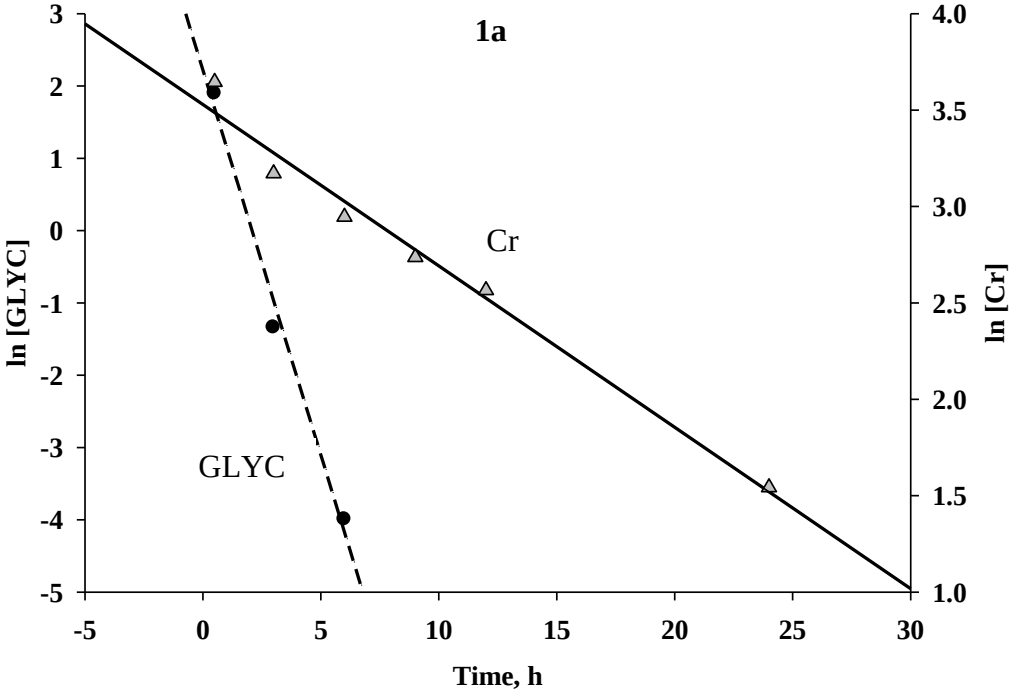
Table 13. Ruminal kinetics of steers ruminally dosed with glycerol and fed high-roughage or high-concentrate diets.

Diet	**Roughage**[1]		**Concentrate**	
n	5		4	
Fluid volume, L	82.56	(9.03)	46.00	(5.05)
Fluid passage rate, %/h	8.39	(1.50)	6.97	(0.95)
Glycerol disappearance rate, %/h	84.11	(4.05)	65.29	(7.63)
Glycerol disappearance rate-fluid passage rate,%/h[2]	75.72	(4.60)	58.32	(6.90)
Estimated escape glycerol, % glycerol intake[3]	10.01	(1.96)	10.69	(0.87)

[1] Mean (S_d).

[2] Estimated rate of fermentation and ruminal absorption of glycerol.

[3] (Fluid passage rate/glycerol disappearance rate) × 100.



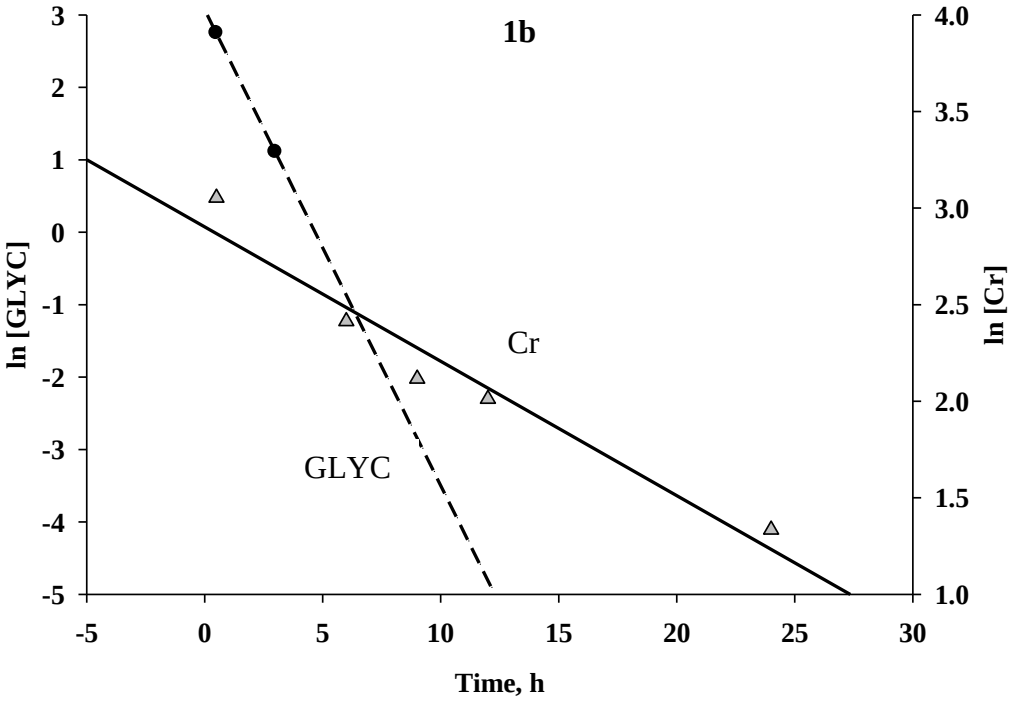


Figure 1a and 1b. Rumen glycerol (GLYC, ●) and chromium (Cr, ▲) concentrations over time in steers ruminally dosed with glycerol and fed low-quality forage (1a) or high-concentrate (1b) diets.

CHAPTER FOUR:

EFFECTIVE LEVEL OF GLYCEROL IN RECEIVING DIETS OF FEEDER CALVES

INTRODUCTION

Newly weaned calves are faced with the stress of physical separation from the dam, deprivation of feed and water during transportation, and adaptation to the feedlot environment. Low intake relative to maintenance can persist for several days post-arrival in the feedlot which challenges the glucose status and health of the calf. Previous research conducted at the SDSU Ruminant Nutrition Center demonstrated that glycerol could be substituted for corn silage to increase intake and growth rate while backgrounding feeder calves. Glycerol is an energy dense commodity that can readily serve as a precursor for *de novo* glucose synthesis. Fermentation of glycerol in the rumen increases production of propionate which is also a glucogenic compound. This experiment was designed to evaluate whether supplemental glycerol will expedite the re-establishment of normal glucose status and energy balance in receiving calves.

MATERIALS AND METHODS

This experiment was conducted at the SDSU Ruminant Nutrition Center from October through December 2014. All experimental procedures used in this study were approved by the South Dakota State University Institutional Animal Care and Use Committee.

Animals and Treatments

Predominantly Angus steer calves (n=225; 306 kg) were weaned and shipped 580 km to the SDSU Ruminant Nutrition Center. Dietary treatments for the 53 d receiving study consisted of 0, 8, 16, and 24% glycerol replacing dry rolled corn in sorghum silage-based diets of similar nutrient composition (Table 15). To normalize protein status as glycerol replaced corn, corn gluten meal was added to offset dietary CP from corn present in the 0% glycerol diet. Calves were allowed to rest overnight before processing with access to long-stem hay and water. The following morning calves were weighed, tagged, vaccinated (Bovi-Shield GOLD 5 and Ultrabac 7/Somubac; Zoetis, Kalamazoo, MI), and treated for parasites (Cydectin; Boehringer Ingelheim, St. Joseph, MO). The processing BW was used to allot steers to 1 of 4 dietary treatments (6 pen replicates per diet; 9 steers per pen) such that BW (n=216; 287 ± 26 kg) was stratified similarly among and within all pens. The second morning in the feedlot steers were weighed and test diets were delivered.

Feed deliveries were managed to accommodate naive calves during the initial 17 d of the receiving phase. Feed was delivered twice daily beginning at 0800 and 1400 h in accordance with clean bunk management. Diets were mixed using a reel-type mixer and feed ingredients were added to the nearest 0.45 kg. All diets were formulated to provide 25

g/ton monensin. Diet changes were made to accommodate differences in CP among dietary treatments (d 8) and to remove grass hay from the diet (d 40).

Diet ingredients were sampled weekly for nutrient analysis. Orts were removed, weighed, and sub-sampled prior to the morning feed delivery for determination of DM. Feed samples were dried in a forced air oven at 60°C for 24 h to determine DM content (AOAC, 1990) and ground through a 1mm screen. Ground feed samples were analyzed for NDF and ADF (Goering and Van Soest, 1970), CP (Kjeldahl procedure; AOAC, 1990), and ash content (AOAC, 1990). Actual diet formulations and compositions were calculated using weekly feed assay data and feed batching records.

Feed records were summarized for each interim period corresponding to weigh days. Individual BW were measured in the morning prior to feed delivery on d 1, 11, 22, and 53 post-arrival. Steers received an implant on d 22 in the feedlot (Synovex-S; Zoetis, Kalamazoo, MI). Following the receiving study, steers were fed a common finishing diet.

Blood Sampling and Analysis

Three steers per pen were randomly selected as sentinel steers for blood collection and were used for sampling throughout the entire study. Samples were acquired 4 h following the first feed delivery on d 6, 20, and 48. Blood was collected via jugular venipuncture using 18 gauge needles and 10 mL tri-potassium ethylenediaminetetraacetic acid vacuum-sealed tubes. Blood was centrifuged at 2,000 × g for 20 min at 4°C. Plasma was sorted into five aliquots and stored in borosilicate glass tubes (13 × 100 mm) at -20°C.

Plasma samples were prepared for analyses by thawing and centrifuging at 430 × g for 10 min at 4°C to separate any coagulated plasma. Plasma non-esterified fatty acid

(NEFA), glycerol, glucose, and urea nitrogen (PUN) concentrations were determined using colorimetric analyses. Quantification of NEFA concentrations involved acyl-CoA synthetase, acyl-CoA oxidase, and peroxidase using 96-well microtiter plates (NEFA-HR (2); Wako Diagnostics, Mountain View, CA). Glycerol was phosphorylated to glycerol-1-phosphate by glycerol kinase and then oxidized to dihydroxyacetone phosphate (DHAP) and hydrogen peroxide by glycerol phosphate oxidase (Free Glycerol Determination Kit; Sigma Aldrich, Saint Louis, MO). Plasma glucose concentrations were determined via glucose oxidase and peroxidase (Liquid Glucose (Oxidase) Reagent; Pointe Scientific, Canton, MI). The PUN assay used was based on the reaction of sodium phenate and sodium hypochlorite as described by Fawcett and Scott (1960). All samples were run in triplicate, allowing 5% variation between the high and low replicates.

Carcass Data Collection

Receiving-backgrounding treatments were balanced among the subsequent finishing phase study treatments and individual steers within pen replicates were re-allotted. Steers were marketed when the majority of the population was estimated to average 1.27 cm in ribfat thickness based on visual appraisal. Steers were co-mingled at 1400 h on d 203 in the feedlot and shipped as a single lot to Tyson Fresh Meats in Dakota City, NE. Individual steer identity was maintained throughout the packing plant and matched to camera grading data acquired from the abattoir.

Statistical Analysis

Treatment and pen replicate were included in the randomized complete block model (GLM, SAS; SAS Inst. Inc., Cary, NC) as independent sources of variation to evaluate the response of dietary glycerol on steer performance using pen as the experimental unit. The model for evaluating the influence of dietary glycerol inclusion and time (repeated measures) on blood metabolites included treatment, pen replicate, and time as independent variables with individual steer as the experimental unit. The effects of glycerol inclusion in receiving diets on carcass characteristics were analyzed on an individual steer basis with receiving treatment, finishing implant strategy, and finishing diet included in the model as independent sources of variation. Differences between means were considered significant at $P \leq 0.05$, and tendencies were reported at $0.05 \leq P \leq 0.10$. Contrast statements were used to determine if relationships between dietary treatment and the dependent variables were linear or quadratic.

RESULTS AND DISCUSSION

Steer Performance

Calves shrunk approximately 6% due to weaning and transportation (1 - (processing BW ÷ pay BW). Increasing levels of dietary glycerol did not affect DMI (Table 16). Although feed deliveries were managed to accommodate naive calves during the initial 17 d of the receiving phase, it is noteworthy that dietary glycerol did not depress intake.

Body weight change and G:F increased linearly ($P<0.05$) during the 1 to 11 d interim period with increasing glycerol inclusion. From d 1 to 6 post-arrival, body weight change of sentinel steers increased linearly with a maximum of 8 kg more than Controls when glycerol was included at 24% of receiving diets (Table 17). Steers fed lower levels of glycerol exhibited linear, compensatory growth during the 12 to 22 d interim period ($P<0.05$). There were no differences in steer performance throughout the remainder of the 53 d receiving phase.

Hales et al. (2013b) included glycerol up to 10% in receiving diets (25% roughage diet; control) replacing grass hay. Contrary to our previous backgrounding study, Hales et al. (2013b) noted a linear decrease in DMI which was partially attributed to an increase in energy density of the diet. Growth rate decreased linearly from d 1 to 28, but cumulative ADG was not different as glycerol replaced roughage during the 42 d receiving phase (Hales et al., 2013b). In contrast to our receiving study, Hales et al. (2013b) provided calves with long-stem wheat hay for up to 5 d post-arrival before treatments were initiated. It was during this 6 d period post-arrival that we observed substantial differences in body weight change as glycerol increased in the diet.

Considering DMI and presumably NE_G intake were similar among treatments throughout our study, the dramatic differences in the pattern of BW gained within 22 d post-arrival are noteworthy. The early weight gain advantage of feeding glycerol is likely due to the glucogenic property of glycerol relative to demand. Intakes were at 1.09 × maintenance by d 3 and at 2.8 × maintenance by d 22. Calves fed the 24% glycerol diet had consumed a total of 4.2 kg of glycerol by d 6 post-arrival. Glycerol fed steers outperformed the Control steers when intake was below or near maintenance. As calves consumed more multiples of maintenance, the influence of dietary glycerol on BW change became negligible.

Plasma Metabolites

Plasma NEFA, glycerol, and glucose concentrations decreased linearly ($P<0.05$) while PUN concentrations increased linearly ($P<0.05$) as glycerol increased in the diet (Table 18). A treatment × time interaction was detected for plasma glucose concentrations ($P<0.05$) with similar levels among treatments on d 6 and 20, and elevated glucose concentrations in Control steers compared to glycerol fed steers on d 48. It is noteworthy that plasma glucose concentrations decreased linearly in finishing steers fed increasing levels of glycerol in a previous study (Chapter 2). Lower NEFA concentrations in glycerol fed steers at d 6, 20, and 48 indicate mobilization of stored triglycerides was minimized. Liu et al. (2014) supplemented glycerol during periods of heat stress to lactating dairy cows and noted lower NEFA concentrations compared to control cows. The observed changes in blood glycerol and glucose levels suggest that higher levels of dietary glycerol may suppress *de novo* glucose synthesis. Elevated PUN concentrations with increasing dietary

glycerol on d 6 and 20 may reflect changes in energy use in the rumen. Glycerol absorption across the rumen epithelium would reduce the amount of fermentable organic matter available for rumen microbes. Considering DIP was similar among treatments, this would result in elevated rumen NH_3-N, and ultimately higher PUN concentrations in glycerol fed steers.

Carcass Characteristics

The influence of glycerol inclusion in receiving diets on carcass characteristics are presented in Table 19. There were no receiving × finishing treatment interactions. Carcass Yield Grade data confirm that these cattle were fed to a common fat endpoint. Dressing percentage, HCW, LMA, ribfat depth, KPH, marbling, and Yield Grade were not affected by treatment (P>0.10). The early weight gain advantage of feeding glycerol during the receiving phase had no impact on Quality Grade.

IMPLICATIONS

Dietary glycerol had no adverse impact on acceptability of feed to incoming, naive calves. Replacement of up to 24% dry rolled corn with dietary glycerol hastened re-establishment of BW, normal glucose status, and energy balance in newly weaned calves introduced into the feedlot environment. The early weight gain advantage of feeding glycerol during the receiving phase had no impact on eventual Yield Grade or Quality Grade. Feedlot size, accessibility of glycerol, and storage availability will dictate practical inclusion levels of dietary glycerol. Based on the observed changes in weight gain and NEFA responses, the inclusion of 16 to 24% glycerol in receiving diets of feeder calves is recommended. Future studies should address whether these responses could lead to improved immune function and health during the receiving phase for more highly stressed calves.

Table 14. Receiving study formulas for pelleted supplement batches.[1]

	Treatment			
Glycerol inclusion, %	**0**	**8**	**16**	**24**
Ingredient	**Kilograms**			
1-7 d				
Ground corn	478	328	178	24
Soybean meal	335	337	341	344
Corn gluten meal	--	141	284	430
Limestone	68	68	68	69
Trace mineralized salt	24	24	24	24
Potassium chloride	--	7	10	14
Microingredients[2]	2	2	2	2
8-53 d				
Ground corn	414	277	138	7
Soybean meal	376	380	383	380
Corn gluten meal	--	124	254	381
Limestone	86	86	87	88
Trace mineralized salt	29	29	30	31
Potassium chloride	--	9	13	18
Microingredients[3]	2	2	2	2

[1] As is basis.

[2] Microingredients included monensin: 186 g, vitamins A & E, zinc hydroxychloride, and tribasic copper chloride.

[3] Microingredients included monensin: 237 g, vitamins A & E, zinc hydroxychloride, and tribasic copper chloride.

Table 15. Receiving diet formulations and compositions.[1]

	Treatment			
Glycerol inclusion, %	**0**	**8**	**16**	**24**
1-7 d				
Grass hay, %	14.65	14.65	14.63	14.63
Sorghum silage, %	33.02	33.01	32.97	32.98
Dry rolled corn, %	32.54	24.95	17.36	9.79
Glycerol, %	--	7.59	15.16	22.75
Dried distillers grains, %	8.43	8.42	8.42	8.42
Pelleted supplement, %[2]	11.36	11.38	11.46	11.43
DM, %	55.29	55.47	55.68	55.82
CP, %	12.06	12.52	12.79	13.23
NDF, %	36.05	35.36	34.65	33.97
NE_M, Mcal/kg[3]	1.72	1.72	1.72	1.72
NE_G, Mcal/kg[3]	1.03	1.03	1.03	1.03
8-39 d				
Grass hay, %	14.46	14.45	14.44	14.43
Sorghum silage, %	30.20	30.18	30.17	30.16
Dry rolled corn, %	36.95	28.91	20.88	12.86
Glycerol, %	--	8.04	16.07	24.08
Dried distillers grains, %	8.94	8.93	8.93	8.92
Pelleted supplement, %[2]	9.45	9.49	9.51	9.55
DM, %	62.55	62.79	63.03	63.28
CP, %	12.47	12.60	12.76	12.83
NDF, %	33.37	32.63	31.90	31.17
NE_M. Mcal/kg[3]	1.75	1.75	1.75	1.75
NE_G, Mcal/kg[3]	1.06	1.06	1.06	1.06
40-53 d				
Sorghum silage, %	46.85	46.73	46.63	46.50
Dry rolled corn, %	37.07	28.94	20.87	12.83
Glycerol, %	--	8.19	16.36	24.48
Dried distillers grains, %	6.53	6.51	6.50	6.48
Pelleted supplement, %[2]	9.55	9.63	9.64	9.71
DM, %	54.41	54.72	54.99	55.30
CP, %	12.55	12.69	12.84	12.93
NDF, %	32.06	31.27	30.48	29.69
NE_M, Mcal/kg[3]	1.73	1.73	1.73	1.73
NE_G, Mcal/kg[3]	1.04	1.04	1.04	1.04

[1] All values except DM on DM basis.

[2] Contains 25 g/T monensin and provides vitamins and minerals to meet or exceed nutrient requirements (NRC, 1996) (Table 14).

[3] Based on tabular NE values of ingredients fed.

Table 16. Interim periods and cumulative steer performance responses to 0, 8, 16, and 24% glycerol in receiving diets.[1]

	Treatment					Contrasts, *P*-value[2]	
	0	**8**	**16**	**24**	**SEM[3]**	**Linear**	**Quadratic**
Initial BW, kg	287	287	287	287	0.315	--	--
1-11 d							
d 11 BW, kg	297	299	301	303	1.1	<0.01	--
ADG, kg	0.96	1.22	1.36	1.56	0.100	<0.01	--
DMI, kg	4.17	4.19	4.20	4.21	0.000	--	--
G:F, g/kg	230	293	324	370	24.0	<0.01	--
F:G	4.41	3.47	3.14	2.81	0.769	<0.01	--
12-22 d							
d 22 BW, kg	325	324	323	323	1.7	--	--
ADG, kg	2.60	2.20	2.00	1.76	0.172	<0.01	--
DMI, kg	7.68	7.75	7.65	7.70	0.065	--	--
G:F, g/kg	338	284	261	228	21.0	<0.01	--
F:G	3.10	3.56	3.88	4.67	0.339	<0.01	--
23-53 d							
d 53 BW, kg	380	376	375	376	2.0	--	--
ADG, kg	1.69	1.63	1.64	1.69	0.060	--	--
DMI, kg	9.15	9.07	8.97	9.15	0.143	--	--
G:F, g/kg	185	180	182	184	6.0	--	--
F:G	5.44	5.56	5.51	5.45	0.166	--	--
Cumulative							
ADG, kg	1.75	1.67	1.66	1.68	0.037	--	--
DMI, kg	7.91	7.87	7.80	7.92	0.092	--	--
G:F, g/kg	221	213	213	212	3.0	0.12	--
F:G	4.54	4.70	4.71	4.72	0.075	0.12	--

[1] Non-shrunk BW basis.
[2] Probability > 0.20 not depicted.
[3] n=6 pens/treatment.

Table 17. Interim periods BW change (kg) of steers fed 0, 8, 16, and 24% glycerol in receiving diets.[1]

	Treatment					Contrasts, *P*-value[2]	
Glycerol inclusion, %	**0**	**8**	**16**	**24**	**SEM**	**Linear**	**Quadratic**
Days	**BW change, kg**						
1-6[3]	1	5	8	9	1.7	<0.01	--
1-11[4]	10	12	14	15	1.0	<0.01	--
12-22[4]	29	24	22	20	1.9	<0.01	--
23-53[4]	54	52	51	54	1.8	--	--

[1] Non-shrunk BW basis.
[2] Probability > 0.20 not depicted.
[3] Sentinel steers (n=3/pen).
[4] All steers (n=9/pen).

Table 18. Blood metabolites over time of steers fed 0, 8, 16, and 24% glycerol in receiving diets.[1]

	Treatment					*P*-value[2]		
	0	**8**	**16**	**24**	**SEM**	**Diet, Linear**	**Day**	**Diet × Day**
Day	**NEFA, mmol/L**							
6	0.1021[a]	0.0921[ab]	0.0890[ab]	0.0729[b]				
20	0.1196[a]	0.1014[ab]	0.0988[ab]	0.0812[b]	0.0085	<0.01	0.08	--
48	0.1151[a]	0.1134[a]	0.0937[ab]	0.0850[b]				
	Glycerol, mg/dL							
6	2.36	2.45	2.09	2.42				
20	2.34[a]	2.15[ab]	2.01[b]	1.97[b]	0.14	0.02	0.08	--
48	2.42	2.51	2.18	2.07				
	Glucose, mg/dL							
6	83.0	79.9	78.9	79.5				
20	76.1	79.6	76.4	74.6	2.5	0.04	0.03	0.04
48	86.2[a]	73.0[b]	70.7[b]	71.8[b]				
	PUN, mg/dL							
6	5.23[a]	5.78[ab]	5.24[a]	6.02[b]				
20	4.55[a]	4.66[a]	5.02[ab]	5.60[b]	0.18	<0.01	<0.01	0.14
48	5.52	5.54	5.24	5.91				

[1] Sentinel steers (n=3/pen).

[2] Probability > 0.20 not depicted.

[a b c] Means within day without a common superscript differ ($P \leq 0.05$).

Table 19. Carcass traits of steers fed 0, 8, 16, and 24% glycerol during the receiving phase.[1]

	Glycerol inclusion, %					Contrasts, *P*-value[2]	
Item	**0**	**5**	**10**	**15**	**SEM**	**Linear**	**Quadratic**
Dress, %[3]	62.61	62.84	62.83	62.73	0.210	--	--
HCW, kg	391	381	386	382	3.2	--	--
LMA, cm^2	92.80	91.76	90.51	90.58	0.815	0.13	--
Ribfat, cm	1.42	1.37	1.40	1.35	0.050	--	--
KPH, %	1.96	1.98	1.97	1.95	0.019	--	--
Marbling[4]	563	560	591	558	8.0	--	0.17
Yield Grade	3.03	2.95	3.07	2.98	0.163	--	--

[1] Individual carcass basis.
[2] Probability > 0.20 not depicted.
[3] HCW as % of shrunk BW.
[4] 400 = Slight°; 500 = Small°.

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