CRUDE GLYCERIN IN FEEDLOT CATTLE DIETS AND AS A SOLVENT IN MAILLARD REACTION PROCESSES INTENDED FOR MANUFACTURING VALUE-ADDED PROTEIN MEALS

by

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Abstract

Two trials were conducted to evaluate effects of crude glycerin, a byproduct of the biodiesel industry, on feedlot performance, carcass characteristics, and diet digestibility in cattle. A third study was conducted to investigate the use of glycerin as a solvent in Maillard reaction processes used to manufacture value added protein meal. In trial 1, crossbred yearling heifers were fed low levels of glycerin (0, 0.5, or 2% of diet DM) in corn finishing diets, or diets that combined corn with soybean hulls and wet distiller's grains (0 or 2% glycerin). Results indicated that feeding glycerin decreased DMI ($P = 0.04$), and feeding byproducts increased DMI ($P < 0.01$) when compared to control without byproducts or glycerin. Feeding byproducts or glycerin decreased the percentage of carcasses that graded USDA Choice or higher ($P < 0.05$). Other live performance traits and carcass characteristics were similar across treatments. Trial 2 evaluated effects of crude glycerin on growth performance and diet digestibility in heifers fed high forage growing diets. Treatments consisted of 0, 4, or 8% crude glycerin added to growing diets containing corn silage (60% of DM) and wet corn gluten feed. Apparent total tract digestibilities were calculated from total fecal collections. Adding glycerin linearly increased ($P = 0.01$) feed efficiency over the entire feeding period, and linearly decreased ($P = 0.02$) DMI for a portion of the feeding period. No other effects of glycerin on animal growth performance were observed. Digestibility measurements indicated that glycerin decreased DM, OM, and NDF intakes linearly ($P < 0.01$), but did not affect fecal outputs of DM, OM, or NDF. Apparent total tract digestibilities of DM, OM, and NDF therefore decreased linearly ($P < 0.01$) with increasing levels of glycerin. The third trial involved several experiments, which were conducted to determine if glycerol could be used as a solvent in processes designed to facilitate non-enzymatic browning of protein meals. Results indicated that glycerol may serve as a more suitable solvent

for browning processes than water because its chemical and physical properties may enhance browning processes, increase process efficiency, and yield products with superior resistance to microbial degradation.

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CHAPTER 1 - Crude glycerin as a component of feedlot cattle diets: a review

C. J. Schneider and J. S. Drouillard

Introduction

Historically, glycerol, or glycerin, was derived from animal fat sources as a byproduct of soap production. When in its pure form, glycerin is a sweet, colorless, odorless, viscous liquid. High purity forms of glycerin are used in a multitude of industrial applications and in food and pharmaceutical industries as a humectant and texturing agent (McGraw-Hill, 2005). The broad range of applications for glycerin once justified production of synthetic glycerin from petroleum. However, production of crude glycerin, a byproduct of the biodiesel industry, has discouraged production of synthetic glycerin in the United States (Niles, 2006). Crude glycerin is among the principal co-products of biodiesel production, comprising approximately 10% (by weight) of the oil that is used to manufacture biodiesel (Dasari et al., 2005). Crude glycerin from biodiesel production lacks the purity to be directly incorporated into many commercial applications, thus requiring further refinement to meet the specifications of various commercial grades.

Expansion of the Biodiesel Industry

The biodiesel industry has expanded exponentially since the introduction of the biodiesel tax credit, which allowed biodiesel prices to be competitive with diesel fuel derived from petroleum (National Biodiesel Board, 2010). This rapid expansion of the biodiesel industry flooded the market with crude glycerin, causing the price to plummet. Many large biodiesel plants began refining their own glycerin to combat low crude glycerin prices, but this solution was not feasible for multitudes of more moderately sized operations that lacked sufficient volume to justify a refinery, and the surplus remained (Niles, 2006). Low market prices of crude glycerin inspired interest in the byproduct by the livestock and poultry industries as a potential carbohydrate source. Expanded utilization of crude glycerin and versatility of more pure forms of

glycerin in various applications will likely continue to increase the value of crude glycerin (Voegele, 2008). Therefore, increased utilization of glycerin by other industries is likely to moderate the use of glycerin in livestock diets. Also, the tax credit for biodiesel expired at the end of 2009, which may lead to a decrease in availability of crude glycerin and a greater increase in glycerin value due to decreased production of biodiesel. Ostensibly, legislation is moving toward the renewal of the tax credit, but despite all efforts of the renewable fuels industry to promote the renewal of the tax credit, many plants remain idle due to unfavorable economic conditions (National Biodiesel Board, 2010). Utilization of glycerin by the livestock industry will be contingent on a favorable glycerin price compared to other carbohydrate sources.

Biodiesel Production Processes

Three basic routes exist for production of alkyl esters from plant oils or animal fat sources, including base-catalyzed transesterification with alcohol, direct acid-catalyzed esterification with methanol, and a two-phase process where oil is first converted to fatty acids and then to alkyl esters using acid catalysis (National Biodiesel Board, 2010). Although the acid catalyzed process is sometimes used as a pretreatment for feedstocks containing a large portion of free fatty acids, typically all biodiesel production facilities utilize base-catalyzed transesterification of the oil with alcohol because it is the most efficient process (Van Gerpen, 2005). This process involves reacting feedstocks with alcohol (usually methanol) in the presence of a catalyst, such as sodium hydroxide or potassium hydroxide, and is relatively efficient because it directly converts feedstocks to alkyl esters in one step, requires lower temperature and pressure than other processes, has higher conversion rates, yet minimizes side reactions and reaction time (National Biodiesel Board, 2010). A 6 to 1 molar ratio of alcohol to oil is utilized in most facilities to drive the reaction to completion, and most of this excess alcohol will remain

in the glycerol portion following the process (Thompson and He, 2006). The glycerin portion resulting from the process is roughly 50% glycerol, with the balance consisting of excess methanol, catalyst, and soaps (Van Gerpen, 2005). Minimally, excess methanol will be removed and recycled, but further refining of crude glycerin will vary depending on the production facility (Thompson and He, 2006). Therefore, proper characterization of physical and chemical properties of crude glycerin is necessary before it can be utilized for animal feed or other applications.

Glycerin Composition

Glycerin is divided into several grades both by the amount of pure glycerol and the impurities present in the glycerin. Although pure glycerol is colorless, less pure forms such as crude glycerin can range in color from a light amber to a very dark brown. This color variation is associated with the variation of impurities present in the glycerin. Impurities in crude glycerin include water, lipid, ash, and some methanol. The ash is mostly residual sodium from the catalyst, which ends up in glycerin along with methanol after the washing step in the biodiesel production process (Thompson and He, 2006). In spite of the popular notion that crude glycerin from biodiesel would fall under the Code of Federal Regulations listing for glycerin (21 CFR 582.1230), which gives glycerin the classification of generally recognized as safe (GRAS), correspondence from FDA early in 2009 stated that crude glycerin from biodiesel was never considered GRAS (Gordon, 2009). The previous author continues by stating that the FDA does not condone use of glycerin from biodiesel in livestock feed unless it contains less than 150 ppm of methanol and meets specifications for glycerin used in pharmaceuticals or food set by U.S. Pharmacopeia and the Food Chemical Code.

Pure glycerol content in crude glycerin derived from biodiesel can vary depending on the

source of the glycerin due to process differences among biodiesel plants (Voegele, 2009). Composition of crude glycerin can be affected by the parent feedstock used in the biodiesel process. Thompson and He (2006) performed nutrient analyses on crude glycerin produced from transesterification of seven different vegetable oil sources and reported that fat, carbohydrate, protein, and ash concentrations varied depending on the type of oil used in the process. The same author also listed ranges for fat content from 1% to 13% and carbohydrate ranges from 75% to 83% for glycerin produced from neat oils, while glycerin derived from waste vegetable oils contained 60% fat and only 27% carbohydrates. Crude glycerin derived from biodiesel production using soybean oil in this study was found to contain 76.2% carbohydrates, 13.04% moisture, 7.98% fat, and 2.73% ash.

Glycerin in Livestock and Poultry Diets

Crude glycerin could have similar feed conditioning effects as molasses when added to animal diets by serving as a texturing agent and possibly by improving palatability due to its sweet nature. It may also improve water holding capacity of rations due to its hydroscopic nature, facilitating dust control. Several researchers have investigated use of glycerin to improve quality of pelleted feeds. Groesbeck et al. (2008) added glycerin at levels ranging from 0 to 15% to the mash of corn-based swine diets and found a linear decrease in energy usage associated with pelleting as glycerin concentrations of the mash were increased. They also evaluated effects of glycerin on pellet durability indices (PDI) and reported a quadratic improvement in pellet durability as glycerin was added. Optimal PDI was attained with 9% glycerin. Similarly, Schröder and Südekum (2007) observed that adding 10% glycerin resulted in the greatest improvement in pellet hardness. The same authors evaluated effects of storage duration on

pellets and noted a preserving effect on concentrate pellets with glycerin concentrations as low as 5%.

Biodiesel-derived glycerin has been utilized effectively as a carbohydrate source for poultry (Simon et al., 1996; Cerrate et al., 2006), pigs (Mourot et al., 1994; Lammers et al., 2008a), sheep (Gunn et al., 2010), and cattle (Schröder and Südekum, 2007). Inclusion levels for crude glycerin in animal diets have typically ranged from 0 to 20% of the diet. Both positive and negative effects on animal performance have been associated with crude glycerin, depending on feeding levels.

Swiatkiewicz and Koreleski (2009) demonstrated 6% crude glycerin could be fed to laying hens without affecting nutrient retention, egg production, or egg quality. Cerrate et al. (2006) found that 10% crude glycerin had a negative effect on feed efficiency of broiler chickens, but 2.5 to 5% glycerin improved feed conversion and daily gain. Lammers et al. (2008a) reported that the energy value of glycerin was approximately equivalent to the energy value of corn in swine diets. Kijora et al. (1995) advocated glycerin levels up to 10% in swine diets, but found higher levels to have deleterious effects on growth efficiency. Though several researchers have reported positive performance effects of adding glycerin, contradictions exist in the literature pertaining to performance of pigs fed crude glycerin. Lammers et al. (2008b) found no effects on ADG or G:F when glycerin was added at 5 or 10% of the diet at any phase of production. In contrast, Groesbeck et al. (2008) fed glycerol at 0, 3, and 6% to nursery pigs and observed a linear improvement in ADG as glycerin concentration increased. Also, Duttlinger et al. (2008) found no effects on ADG or G:F in finishing pigs when glycerin was fed at 2.5 or 5%, but another study performed by Duttlinger et al. (2009) found adding 5% glycerin tended to improve G:F. Gunn et al. (2010) added 0, 5, 10, 15 or 20% crude glycerin to diets of finishing

wethers and noted linear increases in DMI and quadratic increases in ADG and G:F during the first 14 d of the feeding period, but found no treatment differences over the entire feeding period. Although contradictions exist in the literature pertaining to potential benefits of adding glycerol, no negative performance effects have been reported for poultry, swine or sheep as a result of including moderate levels of crude glycerin to the diet.

The majority of the early scientific literature pertaining to glycerin usage in cattle evaluated the potential for glycerin as a glucogenic supplement to prevent ketosis in dairy cattle. However, a portion of the literature would suggest that glycerin supplementation has no positive effect in attenuating incidence of ketosis and may actually increase severity of ketosis (DeFrain et al., 2004; Ogborn, 2006). Only a small portion of the literature pertains to the effects of adding crude glycerin to finishing cattle diets. Therefore, little is understood about possible interactions between methods of grain processing, roughage type, or other feed additives and glycerin, which could explain contradictions that exist in the early literature. Mach et al. (2009) replaced 0, 4, 8, or 12% barley grain with crude glycerin in high concentrate diets containing ground corn for Holstein bulls and observed no differences in DMI, ADG, or G:F. In contrast, Parsons et al. (2009) evaluated 0, 2, 4, 8, 12, or 16% crude glycerin in steam-flaked corn finishing diets for feedlot heifers and found that DMI, ADG, and feed efficiency all responded quadratically to glycerin concentration. Performance was optimized when glycerin was added at 2% of the diet DM, and glycerin greatly depressed DMI (relative to controls) at levels of 12% and higher. Similarly, Pyatt et al. (2007) reported a 10% reduction in DMI and a 19% improvement in efficiency when 10% crude glycerin was added to feedlot diets containing either 70% rolled corn with 10% distiller's grains or the combination of 35% rolled corn, 30% distiller's grains, and 15% soybean hulls. Elam et al. (2008) fed 0, 7.5, or 15% crude glycerin and observed a linear

reduction in DMI and no change in efficiency. Notably, glycerin has been shown to increase the percentage of carcasses grading USDA Select at the expense of them grading USDA Choice, despite glucogenic properties of glycerin (Elam et al., 2008; Parsons et al., 2009). Collectively, these studies suggest that although glycerin may decrease DMI and quality grade, modest amounts of crude glycerin can be added to feedlot diets without deleterious effects on performance. However, improvements in gain or efficiency as a result of adding glycerin may be influenced by other factors that are not are not well understood.

Ruminal Metabolism of Glycerol

Glycerin is rapidly fermented to VFA in the rumen (Wright, 1969; Rémond et al., 1993). However, reports of the end products of glycerin fermentation by rumen microflora have been somewhat controversial. Reports in the early literature suggested fermentation of glycerin yielded almost entirely propionate (Johns et al., 1953; Garton et al., 1961). Additional reports suggest increases in both propionate and butyrate as a result of glycerin addition (Czerkawski and Breckenridge, 1972; Ferraro et al., 2009). Bergner et al. (1995) reported most of glycerin is converted to propionate whereas no glycerin was converted to acetate when evaluating fermentation of radio-labeled glycerin by ruminal microbes. Similarly, Trabue et al. (2007) found glycerol somewhat suppressed acetate production in a mixed culture inoculum obtained from a dairy cow fed a 50% concentrate diet. Other *in vitro* and *in vivo* studies have reported the production of propionate at the expense of acetate (Rémond et al., 1993; Kijora et al., 1998). In contrast, Wright (1969) performed an *in vitro* experiment using an inoculum from cattle grazing clover and ryegrass pastures and found that radio-labeled glycerin was converted to acetate, propionate, and butyrate. Parsons and Drouillard (2010) performed an *in vivo* study where 0, 2, or 4% crude glycerin was added to high concentrate diets and noted linear decreases in

concentrations of butyrate, valerate, and acetate as glycerin level increased, whereas propionate concentrations were unaffected. Another researcher determined *Klebsiella planticola*, a strain isolated from ruminal contents of red deer, converted glycerol into approximately equal proportions of formate and ethanol (Jarvis et al., 1997). Collectively, the variation in end products of glycerin metabolism found in the scientific literature would suggest that glycerin metabolites are a direct function of the microflora present in the rumen, which is related to diet type.

Effects of Glycerin on Ruminal Fermentation

Negative effects of glycerin on protein metabolism have been observed in both *in vitro* and *in vivo* experiments. Paggi et al. (1999) added glycerin to the media in concentrations ranging from 50 mM to 300 mM of glycerin and found that glycerin decreased relative proteolytic activity of rumen fluid by approximately 20% at all concentrations evaluated. The authors concluded glycerin may make the media less suitable for proteolytic enzyme activity because glycerin lacks a hydrophobic chain. Similarly, Kiljora et al. (1998) ruminally dosed 200 g of glycerol (10% of DMI) twice daily over a period of 6 d and noted decreases in branched-chain VFA concentrations and bacterial protein synthesis, suggesting glycerol decreased protein degradation and growth by ruminal bacteria.

Glycerin has also been observed to affect cellulolytic activity in the rumen. Roger et al. (1992) found that *in vitro* degradation of cellulose by ruminal fungi was inhibited with glycerol at 0.5%, and cellulolytic bacteria were inhibited when glycerol concentrations were at 5% of the media. Likewise, Paggi et al. (2004) evaluated the effects of glycerin on IVDMD of oat hay and carboxymethyl-cellulose (CMC), which is a soluble substrate that is less complex than forage, and noted that glycerin decreased IVDMD of both substrates equally. However, inhibitory

effects on total cellulolytic activity in this study were noted when glycerin concentrations were approximately equivalent to the level previously shown to inhibit ruminal fungi, and concentrations were well below the concentration reported to inhibit cellulolytic bacteria by Roger et al. (1992). These *in vitro* studies are in accordance with the findings of Parsons and Drouillard (2010), who observed decreases in apparent total tract digestibilities of NDF in animals consuming high concentrate diets with increasing levels of crude glycerin. Though fiber is normally not a major component of feedlot diets, suppression of cellulolytic activity by glycerin could potentially alter performance of animals fed diets containing greater concentrations of fiber, such as byproduct-based diets or forage-based diets.

Summary

Renewal of the biodiesel tax credit would likely result in continued expansion of the biodiesel industry, further increasing availability of crude glycerin. However, glycerin surpluses have greatly expanded glycerin usage and if surpluses decline, higher value applications for glycerin may discourage glycerol use as livestock feed. Even so, glycerin has been utilized effectively as an energy source by the livestock industry. Several feedlot studies have reported significant performance improvements as a result of feeding glycerin. Glycerin has been shown to increase the number of carcasses grading USDA Select at the expense of those grading USDA Choice and can have negative effects on fiber digestion. Much remains to be understood about metabolism of glycerol and possible dietary interactions that can affect performance of feedlot cattle.

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CHAPTER 2 - Prevention of ruminal protein degradation by non-enzymatic browning with glycerol as a potential solvent: a review of the literature

C. J. Schneider and J. S. Drouillard

Introduction

Historically, non enzymatic browning (NEB) has had a negative connotation with respect to animal feeds because it can decrease bioavailability of amino acids (Boctor and Harper, 1968; Goering et al., 1973). This connotation was disputed after a report suggested some intermediates of the reaction were biologically available to rats (Finot et al., 1977), which led to further investigation of the NEB process as a potential method to prevent protein degradation by ruminal microbes. This review will focus on the Maillard reaction, its implications in manufacturing value added protein sources for ruminants, and the potential for improving existing processes designed to manufacture non-enzymatically browned protein meals by utilizing glycerol.

Maillard Reaction

Non-enzymatic browning (NEB), or the Maillard reaction, has been widely researched by food chemists because of the ubiquitous nature of the reaction in food products and its ability to influence food flavor and shelf life. However, much remains to be understood about the reaction due to its complexity. The Maillard reaction was named after Louis Maillard, the French chemist who first described the reaction when he heated a solution of glucose and lysine and observed formation of brown pigments, or melanoidins. This reaction typically occurs in foods that contain both reducing sugars and amino acids or proteins (Mauron, 1981). Hodge (1953) elaborated on the description of the complex series of reactions involved in the Maillard reaction by dividing it into three stages: initial, intermediate, and final. The initial stage leaves products colorless and involves sugar-amine condensation, yielding Schiff's bases and subsequently glucosylamine, which then undergoes Amadori rearrangement to form Amadori compounds. During the intermediate stage, color changes begin to develop, leaving products yellow. This stage is characterized by dehydration and fragmentation of sugars and Strecker degradation of

amino acids. The final stage involves aldol condensation, aldehyde-amine polymerization, and formation of heterocyclic nitrogen compounds, yielding end products that are highly colored. It is important to note that Mauron (1981) referred to these three stages as early, advanced, and final because this terminology frequently is used in other scientific literature.

Many factors can influence the course of the Maillard reaction, affecting both rate and extent of browning. Temperature and duration of heating are among the most important factors affecting the reaction. Lea and Hannan (1949) found that increasing the temperature from 0 to 80°C caused the loss in amino-nitrogen to occur 40,000 times faster in a reaction involving glucose and casein. Water activity (α_w) and moisture content also have been shown to influence kinetics of the Maillard reaction. Intermediate α_w ranges (0.3-0.7) are optimal for browning reactions in most instances because low α_w will decrease mobility of the reactants, and high α_w can lead to the dilution of the reactants, both resulting in decreased reaction rates (Eichner and Karel, 1972). Reaction rates are also affected by pH, with acidic conditions having an inhibitory effect and alkaline conditions accelerating reaction rates (Lea and Hannan, 1949). All of the previously mentioned factors are frequently used in a variety of industrial applications to induce or attenuate the Maillard reaction.

Use of Maillard Reactions to Prevent Proteolysis in the Rumen

Controlled Maillard reactions can be effectively utilized to produce value added feed products that contain proteins which are resistant to degradation by ruminal microbes (Can and Yilmaz, 2002; Cleale et al., 1987a,b; Nakamura et al., 1992). Most of the scientific literature pertaining to this topic involves processes where soybean meal is heated in the presence of reducing sugars to promote a NEB reaction. Cleale and colleagues (1987a) were the first to apply this concept as a method to decrease ruminal degradation of soybean meal protein. They also

evaluated the effects of several process variables on ammonia release, including reducing sugar type (xylose, glucose, fructose, or lactose), reducing sugar level, product DM, and duration of heating. Xylose was determined to be the most reactive reducing sugar, yielding browned soybean meal products with superior resistance to protein degradation by ruminal microbes. Increasing sugar level and heating time both led to linear decreases in ammonia release, and product DM greater than 80% reduced non-enzymatic browning reaction rates. They concluded controlled NEB was an effective method to prevent degradation of soy protein by ruminal microbes. Similarly, Cleale et al. (1987b) conducted an *in vivo* study and found feeding browned soybean meal increased dietary protein flow to the intestine. Although one trial revealed NEB of soybean meal decreased total tract digestibility of N, a second trial conducted by the same researchers found that proteins from browned soybean meal were more efficiently utilized by lambs than proteins from untreated soybean meal (Cleale et al., 1978 c). Likewise, NEB of soybean meal has been shown to improve efficiency of protein utilization in dairy cattle. (Nakamura et al.,1992). Consequently, this reaction has been exploited commercially to produce protein meals with improved nutritional characteristics for ruminant animals.

Although most of the scientific literature pertains to the direct addition of reducing sugars to promote NEB, several methods have been developed to provide reducing sugars for NEB and generally can be divided into 3 separate processes: 1. Direct addition of the reducing sugars, usually xylose because it is the most reactive (Cleale et al., 1987a) or other commercial sources of xylose such as sulfite liquor (Nakamura et al., 1992); 2. Addition of a carbohydrase enzyme which, under the proper conditions, will convert sugars within soybean meal to reducing forms (Coetzer, 2000); 3. Introduction of an organism capable of producing a carbohydrase enzyme such as *Saccharomyces cerevisiae* (Drouillard and Coetzer, 2003). As previously mentioned,

Cleale et al. (1987a) found that rates of NEB were reduced when product DM exceeded 80%. Therefore all of the previously described methods of providing the reducing sugar have been implemented in production processes where water is added serve as a solvent and prevent a reduction in reaction rates. Excess water must then be removed to prevent mold growth in finished products.

Glycerol as a Solvent for NEB Reactions

One method for reducing cost of NEB processes would be removal of the drying process by eliminating water addition during processing. Glycerol, or even crude glycerin, may serve as a suitable replacement for water in NEB processes. Glycerol is the simplest trihydric alcohol and, when pure, is a colorless, viscous liquid with a vaporization point of 290°C (McGraw-Hill, 2005). Less pure forms such as crude glycerin retain many of the same properties as glycerol but also contain impurities such as water, ash, lipid, and methanol. High purity forms of glycerin are commonly used in a multitude of industrial applications and in food and pharmaceutical industries because the chemical and physical properties of glycerol make it ideal for use as a humectant, solvent, or texturing agent (McGraw-Hill, 2005). Several researchers in the food industry have investigated the effects of humectants such as glycerol on NEB of food products and found that glycerol may increase rate and extent of NEB (Mustapha et al., 1998; Sherwin and Labuza, 2003; Cherny and Guntz, 2006). Addition of glycerol to food model formulations containing sodium caseinate and glucose increased rates of Maillard reactions that occur naturally over time as much as 1.5 fold compared to control formulations without glycerol, suggesting operation of a solvent mechanism (Sherwin and Labuza, 2003). Similarly, Mustapha and co-workers (1998) found that although the reactants were not entirely soluble in glycerol, more extensive browning of lysine and xylose mixtures occurred in glycerol than in an aqueous

solution. Other research suggests that glycerol may serve as a precursor in the Maillard reaction (Cherny and Guntz, 2006), and that heating a glycerol and amino acid mixture even in the absence of reducing sugars will result in a certain degree of browning (Obanu et al., 1977). Bello and Bello (1976) suggested that impurities formed by mild oxidation of glycerol in air may be responsible for NEB of proteins when only glycerol is present.

Addition of glycerin could also prevent the reductions in reaction rates of products above 80% DM. Adding glycerin to products containing little moisture will increase NEB reaction rates because the plasticizing effect of glycerol improves the mobility of the reactants (Eichner and Karel, 1972). Similarly, Labuza and Saltmarch (1981) reported that glycerol will lower the optimum α_w for the maximum rate of browning. For instance, it could move the optimum α_w from 0.7-0.5, meaning that less water activity is required to achieve the maximum rate of browning. Glycerin may also serve as a more economical solvent than water in these processes due to the higher vaporization point of glycerol. Utilizing glycerol in NEB processes could reduce evaporation during the heating process. Reduced evaporate losses in glycerin products could decrease thermal energy requirements for processing as evaporative losses constitute a loss of energy in a production system.

Summary

The Maillard reaction describes an extremely complex series of chemical reactions that occur widely in nature. Food chemists have extensively studied the Maillard reaction in an attempt to understand and control the occurrence of reaction between a reducing sugars and proteins or amino acids. Controlled non-enzymatic browning processes have been effectively utilized to manufacture value-added feed ingredients with proteins that are resistant to ruminal degradation. These value added products increase the effectiveness of protein utilization in

ruminants. Utilization of glycerol in NEB processes designed to protect proteins from ruminal degradation may further enhance existing browning processes due to the plasticizing effect and higher vaporization point of glycerol.

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CHAPTER 3 - Effects of low levels of crude glycerin with or without other co-products on performance and carcass characteristics of feedlot heifers

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Abstract

A trial was conducted to evaluate finishing performance and carcass traits of heifers (n=295; 427 kg BW; SEM 8.8 kg) fed low levels of glycerin (0, 0.5, or 2% of diet DM) in corn finishing diets, or diets that combined corn with soybean hulls and wet distiller's grains (0 or 2% glycerin). Diets contained corn with 3% alfalfa hay and 6% corn silage, and provided 300 mg monensin, 90 mg tylosin, and 0.5 mg melengestrol acetate per heifer daily. In the co-product diets, 25% soybean hulls and 15% wet distiller's grains (DM basis) replaced steam-flaked corn. Cattle were stratified by body weight and randomly assigned (within strata) to 40 concrete-surfaced pens containing 7 to 8 cattle per pen, with 8 pens per treatment. Cattle had *ad libitum* access to feed and water and were fed once daily for 89 d. Feeding 0.5% or 2% glycerin decreased DMI (0.5% $P = 0.06$ and 2% $P = 0.07$) when compared to control cattle receiving no glycerin. Feeding co-products increased DMI when compared to diets without co-products ($P < 0.01$). ADG was not affected by diet ($P > 0.30$; 1.34, 1.22, 1.16 kg/d for cattle fed 0, 0.5, and 2% glycerin without co-products, respectively, and 1.30 and 1.19 kg/d for heifers fed diets with co-products diets containing 0 or 2% glycerin). No treatment differences for G:F were identified ($P > 0.2$). In addition, adding glycerin to diets without co-products decreased the percentage of carcasses that graded USDA Choice or better (Lin; $P = 0.03$), and increased the percentage of carcasses that graded USDA Select (Lin; $P = 0.02$). Similarly, addition of co-products decreased the percentage of carcasses that graded USDA Choice or better and increased the percentage of carcasses that graded USDA Select ($P < 0.05$). Feeding low levels of glycerin yielded no improvements in feedlot performance, decreased DMI, and decreased the percentage of carcasses grading USDA Choice.

Key Words: Glycerin, Wet distiller's grains, Steam-flaked corn

Introduction

Expansion of the renewable fuels industries has increased availability of co-products that are well-suited for use as cattle feed. Glycerin is among the principal co-products of biodiesel production, comprising approximately 10% (by weight) of the soybean oil that is used to manufacture soy-based diesel fuel (Dasari et al., 2005). Previous research evaluated effects of glycerin ranging from 0 to 16% of flaked corn finishing diets, and revealed that optimal growth performance was achieved with 2% glycerin addition (Parsons et al., 2009).

Distiller's grains and other co-products are becoming increasingly common in feedlot rations. Very little research has evaluated the addition of glycerol to diets containing other co-products. Distiller's grains can contain as much as 10% glycerin (Wu, 1994). Feeding excesses of glycerin can decrease feed intake (Parsons et al., 2009). Also, coproduct diets contain appreciable amounts of fiber when compared to grain-based diets. Roger et al. (1992) found that glycerol inhibited in vitro degradation of cellulose by rumen fungi when added at 0.5% and inhibited cellulolytic bacteria when glycerol concentrations were at 5% of the media. Though fungi do not play a vital role in ruminal fermentation of high concentrate diets, the suppression of cellulolytic activity by glycerin may alter performance or reduce DMI of animals fed diets containing more fiber. Feeding more modest amounts of glycerin in coproduct diets may be more practical, and more beneficial. The objective of this research was to evaluate effects on performance and carcass characteristics of finishing cattle fed low levels of glycerin in corn-based finishing diets, as well as in diets that consisted of a combination of corn grain, distiller's grains, and soybean hulls.

Materials and Methods

Finishing Trial

Use of animals in this experiment was approved by the Kansas State University Institutional Animal Care and Use Committee. Crossbred heifers (n=295; initially 427 kg BW; SEM 8.8 kg) were used in a randomized complete block design. Incoming cattle were allowed free access to ground alfalfa hay and were processed within 24 h of arrival. During processing, heifers were identified with an individual ear tag, individually weighed, implanted with Revalor 200 (Intervet, Inc., Millsboro, DE), vaccinated with Bovishield-IV and Fortress-7 (Pfizer Animal Health, Exton, PA), injected with Micotil (Elanco, Greenfield, IN), and orally drenched with Safe-Guard (Intervet, Inc.) for internal parasites. Four weeks subsequent to initial processing, cattle were revaccinated with Bovishield-IV. Prior to initiating finishing treatments, cattle were fed a series of step-up rations to gradually adapt them to their final finishing rations. At the end of the step-up phase, cattle were stratified by BW and randomly assigned (within strata) to 40 pens containing 7 to 8 animals per pen, with 8 pens per treatment. Pens were concrete surfaced (36 m^2) and had overhead shade covering one-half of the pen and the entire feed bunk. Feed bunks provided 3.2 linear m of bunk space, and fence line water fountains were shared between two adjacent feedlot pens. Cattle were given *ad libitum* access to treatment diets, which were fed once daily at approximately 1100 h. Dietary treatments (Table 3-1) were based on corn and consisted of 0, 0.5, or 2% crude soy-based glycerin in grain-based diets, and 0 or 2% crude glycerin in diets containing co-products. Crude glycerin was analyzed by a commercial laboratory (SDK laboratories, Hutchinson, KS) as follows: moisture by Karl Fischer titration according to official method 966.2 (AOAC, 1995); ash using official method 942.05 (AOAC, 1995); Na using official method 956.01 (AOAC, 1995); N using official method 920.176

(AOAC, 1995); and methanol using official method 973.23 (AOAC, 1995). Glycerin contained 14.3% moisture, 6.68% ash, 2.58% Na, 0.04% N, and less than 0.01% methanol. In co-product diets, 25% soybean hulls and 15% wet distiller's grains replaced corn and soybean meal. Composite samples of wet distiller's grains and corn silage were analyzed by a commercial laboratory (SDK laboratories, Hutchinson, KS) for glycerol content by HPLC according to official method 982.22 (AOAC, 1995). Corn silage contained less than 0.1% glycerol and wet distiller's grains contained 7.2% glycerol on a DM basis. Diets were based on dry-rolled corn for the first 37 d of the feeding period, then gradually transitioned to diets based on steam-flaked corn. All diets contained 3% alfalfa hay and 6% corn silage, and provided 300 mg monensin, 90 mg tylosin, and 0.5 mg melengestrol acetate (Elanco Animal Health, Greenfield, IN) per heifer daily. Heifers were also fed zilpaterol HCl (Intervet Inc., Millsboro, DE) at a level of 8.33 mg/kg DM for 21 d followed by a 3-d withdrawal prior to harvesting the animals. Feedstuff samples were collected weekly throughout the duration of the feeding period and analyzed for DM and nutrient content. Dry matter was determined by drying feedstuffs at 105°C for 16 h in a forced air oven. Crude protein was determined using a Leco FP-2000 N analyzer (Leco Corporation, St. Joseph, MI). Lipid content was determined using Gold-fisch ether extraction method. Analyses of NDF were performed according to procedures described by Van Soest et al. (1991) using an ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY). Weight of each pen of heifers was determined at the beginning of the experiment and immediately prior to harvesting the animals on d 89. Cattle were transported to a commercial abattoir in Holcomb, KS on d 89 for harvest. Hot carcass weights and incidence and severity of liver abscesses were recorded on the day of harvest. USDA quality grade; USDA yield grade; marbling score; 12th

rib fat thickness; LM area; and kidney, pelvic, and heart fat were recorded after a 48-h period of chilling.

Statistical Analysis

Performance and carcass characteristics were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Pen was the experimental unit and the random effect was block. The model statement included effects of glycerin, byproduct, and glycerin × byproduct. Percentages of USDA yield grade, USDA quality grade, and liver abscesses were calculated and analyzed using the MIXED procedure of SAS with pen as the experimental unit and block as the random effect. The model statement included effects of glycerin, byproduct, and glycerin × byproduct. Orthogonal contrasts were used to evaluate linear and quadratic effects of glycerin and effects of byproducts versus no byproducts which compared grain-based diets to diets containing soybean hulls and distiller's grains. Orthogonal contrasts were protected by requiring an F-test with $P < 0.1$. Because glycerol is present in wet distiller's grains and therefore present in all byproduct diets, linear and quadratic contrasts to evaluate effects of glycerin level were only performed on grain-based diets.

Results and Discussion

Finishing Performance

Performance data are summarized in Table 3-2. Feeding 0.5% or 2% glycerin in grain diets tended to decrease (0.5%, $P = 0.06$; 2%, $P = 0.07$) DMI when compared to the control steam-flaked corn diet. Previous research from Pyatt et al. (2007) and Parsons et al. (2009) also reported decreases in DMI as a result of feeding glycerin, but these decreases occurred when glycerin was fed at higher percentages of the diet compared to the present study. No effects on

DMI were observed by Parsons et al. (2009) when glycerin was added at 2% in a steam-flaked corn diet. Similar glycerin effects on DMI were not observed in our study ($P > 0.10$) when glycerin was fed in a co-product diet, which may refute the hypothesis that reduction of cellulolytic activity would be more apparent and possibly reduce DMI when feeding diets containing higher fiber. This is in contrast to observations of Pyatt et al. (2007), who reported adding 10% glycerol to co-product diets decreased DMI by 11.8%. In our study, addition of co-products with or without glycerin, increased ($P < 0.01$) DMI when compared to diets without co-products. Although no significant interaction was present, increases in DMI were larger among diets containing glycerol than diets without glycerol ($P < 0.01$; 11.1% increase in DMI among glycerol diets vs. $P < 0.01$; 5.3% increase in DMI among diets without glycerol). Similarly, Depenbusch et al. (2009a) found that including dried distiller's grains with solubles (DDGS) at a level of 15% of diet DM resulted in an increase in DMI when compared to diets containing no DDGS. Increases in DMI could be attributed to the reduction in total starch of the diet as a result of replacing steam-flaked corn with distiller's grains.

Feeding glycerin numerically decreased final BW, ADG and G:F. However, due to large variability among cattle responses to glycerin feeding, final BW, ADG, and G:F of cattle receiving glycerin in grain-based or co-product diets were not different ($P > 0.2$) from cattle receiving 0% glycerin in grain-based or co-product diets ($P > 0.2$). Although no statistical significance was detected, feeding 2% glycerin resulted in a 16 kg decrease in final BW when compared to control cattle receiving no glycerin and similar observations are present for ADG and G:F. The inability of this experiment to detect significance in such a large difference in final BW may suggest the possibility of type II error. Other research pertaining to effects of glycerin on ADG and G:F has been inconsistent. Mach et al. (2009) who replaced barley grain for 0, 4, 8,

or 12% crude glycerin in high concentrate diets containing ground corn for Holstein bulls and observed no differences in ADG or G:F. However, another study indicated that adding glycerol at 2% of diet DM resulted in a 12.6% increase in ADG in feedlot cattle (Parsons et al., 2009). Additionally, Pyatt et al. (2007) reported an 11.7% improvement in ADG when 10% glycerin replaced a portion of the dry-rolled corn in grain diets. Similar contradictions exist for performance effects in studies involving addition of glycerin to pig diets. Lammers et al. (2008) found no effects on ADG or G:F when glycerin was added at 5 or 10% of the diet at any phase of production. In contrast, Groesbeck et al. (2008) fed glycerol at 0, 3, and 6% to nursery pigs and observed a linear improvement in ADG as glycerin concentration increased. Also, Duttlinger et al. (2008) found no effects on ADG or G:F in finishing pigs when glycerin was fed at 2.5 or 5%, but another study performed by Duttlinger et al. (2009) found adding 5% glycerin tended to improve G:F.

No performance effects were observed as a result of adding co-products to the diet. This is in concurrence with the findings of Daubert et al. (2005) who suggested 15% as the optimal inclusion level for wet distillers grains in steam-flaked corn diets, and other studies where feeding distillers grains near 15% did not change G:F (Depenbusch et al., 2008, 2009b).

Carcass Characteristics

Carcass characteristics for heifers are summarized in Table 3-3. No treatment differences were observed for HCW ($P \geq 0.42$) or LM area ($P \geq 0.39$). This is in contrast to the observation of Parsons et al. (2009), who reported an increase in HCW and LM area when glycerol was added at 2% of the diet DM. Dressed yields were not different ($P > 0.30$) regardless of glycerin level or addition of co-products to the diet.

Though no treatment differences ($P \geq 0.64$) were identified for marbling score, addition of glycerin to grain diets linearly decreased ($P = 0.03$) the percentage of carcasses that graded USDA Choice or better, and there was a concomitant linear increase ($P = 0.02$) in the percentage of carcasses that graded USDA Select. Previous research reported decreases in marbling scores and a linear tendency to reduce USDA quality grades with glycerin addition to diets (Parsons et al., 2009). Addition of co-products decreased ($P = 0.02$) the percentage of carcasses that graded USDA Choice or better and increased ($P = 0.02$) the percentage of carcasses that graded USDA Select. Decreases in quality grade when byproducts were added to the diet could conceivably be due to glycerin, as wet distiller's grains contain glycerol. Similar decreases in USDA Quality grade were observed by Depenbusch et al. (2009a), who found the percentage of USDA Select carcasses increased linearly as the level of DDGS in the diet increased. Liver abscess prevalence and percentage of KPH were not affected by treatment ($P > 0.8$). No treatment differences ($P \geq 0.3$) occurred for 12th rib fat thickness or USDA yield grade, which is in contrast to observations of Parsons et al. (2009), who found that feeding glycerol led to a reduction in subcutaneous fat and lower numerical yield grades.

Responses to feeding glycerin in this study were very different from previous studies. Adding low levels of glycerin to grain diets caused a reduction in DMI. Feeding co-products such as wet-distillers grains and soybean hulls increased DMI but did not affect performance. Addition of glycerin or byproducts led to a decrease in the percentage of animals grading USDA choice or better. No statistical significance was detected in numerical decreases in final BW, ADG, or G:F as a result in feeding glycerin. However, the possibility of type II error in this experiment, suggesting the potential for negative performance effects of glycerin, should be considered before utilizing glycerin in a commercial setting. Further characterization of crude

glycerin to evaluate possible negative effects on animal performance caused by glycerol or other contaminants found in crude glycerin will be necessary to explain contradictory effects of glycerin on feedlot performance or identify optimal feeding levels if they exist.

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Table 3-1. Composition of steam-flaked corn finishing diets containing low levels of crude glycerin or diets containing co-products with or without crude glycerin fed to yearling heifers

	Grain-based Diets			Byproduct-based Diets	
Item	0% Glycerin	0.5% Glycerin	2% Glycerin	0% Glycerin	2% Glycerin
Ingredients, % of DM					
Corn	80.6	80.0	78.2	46.6	44.2
Soybean hulls	-	-	-	25.0	25.0
Wet distiller's grains	-	-	-	15.0	15.0
Corn silage	6.0	6.0	6.0	6.0	6.0
Soybean meal	4.4	4.5	4.8	-	0.4
Alfalfa hay	3.0	3.0	3.0	3.0	3.0
Crude glycerin	-	0.5	2.0	-	2.0
Limestone	1.7	1.7	1.7	1.4	1.4
Urea	1.2	1.2	1.2	0.4	0.4
Vitamin/mineral premix[1]	0.9	0.9	0.9	0.4	0.4
Feed additive premix[2]	2.2	2.2	2.2	2.2	2.2
Analyzed Composition, %					
DM	76.1	76.2	76.3	64.6	64.7
CP	14.4	14.4	14.4	14.1	14.1
NDF[3]	11.9	11.9	11.8	28.9	28.8
Crude fat[4]	2.5	2.5	2.5	6.6	6.6
Calcium	0.7	0.7	0.7	0.9	0.9

[1]Formulated to provide 0.1 mg Co, 10 mg of Cu, 0.6 mg of I, 60 mg of Mn, 0.25 mg Se, 60 mg Zn, 2640 IU vitamin A, and 11 IU vitamin E per kg diet DM.
[2]Feed additive premix provided 300 mg of monensin (Elanco Animal Health, Greenfield, IN), 90 mg tylosin (Elanco), and 0.5 mg of melengestrol acetate (Pfizer Animal Health, Exton, PA) per animal daily in a ground corn carrier. Zilpaterol HCl (Intervet Inc., Millsboro, DE) was fed for 21 d before harvest at the rate of 8.33 mg/kg of diet DM, followed by a 3-d withdrawal period.
[3]NRC (2000) feed library NDF values for soybean meal were used in calculation of NDF content.
[4]NRC (2000) feed library fat values for soybean meal and soybean hulls were used in calculation of crude fat content.

Table 3-2. Performance for yearling heifers fed finishing diets based on steam-flaked corn containing 0, 0.5, or 2% glycerin or diets containing byproducts with 0 or 2% glycerin

	Grain-based Diet			Byproduct Diet		
Item	0% Glycerin	0.5% Glycerin	2% Glycerin	0% Glycerin	2% Glycerin	SEM
No. of pens (heifers)	8 (61)	8 (58)	8 (58)	8 (59)	8 (59)	-
Days on feed	89	89	89	89	89	-
Initial weight, kg	427	427	427	428	426	8.8
Final weight, kg	547	536	531	543	532	9.6
Dry matter Intake, kg/day	8.8[a]	8.5[b]	8.5[b]	9.3[bc]	9.5[bc]	0.18
Average daily gain, kg/day	1.34	1.22	1.16	1.30	1.19	0.068
G:F, kg/kg	0.152	0.145	0.137	0.140	0.127	0.0075

[a-c]Within a row, means without a common superscript tend to differ ($P < 0.1$).

Table 3-3. Carcass characteristics for yearling heifers fed finishing diets based on steam-flaked corn containing 0, 0.5, or 2% glycerin or diets containing byproducts with 0 or 2% glycerin

	Grain-based Diet			Byproduct Diet			Contrast *P* – values[1]	
Item	0% Glycerin	0.5% Glycerin	2% Glycerin	0% Glycerin	2% Glycerin	SEM	Glycerin Linear	Byproduct Effect
Hot carcass weight, kg	357	352	350	350	352	5.4	-	-
Dressed yield, %	65.4	65.7	65.9	64.5	66.1	0.58	-	-
LM area, cm^2	91.9	91.7	89.7	89.2	91.2	1.32	-	-
12^{th} rib fat thickness, cm	1.31	1.26	1.28	1.41	1.20	0.084	-	-
KPH, %	2.10	2.02	2.11	2.05	2.10	0.076	-	-
Marbling[2]	450	430	420	430	430	13.1	-	-
USDA yield grade (YG),	2.08	2.09	2.09	2.24	2.08	0.10	-	-
YG 1, %	24	21	22	20	23	4.6	-	-
YG 2, %	45	50	47	39	52	6.8	-	-
YG 3, %	29	27	31	38	20	6.9	-	-
YG 4, %	2	2	0	3	5	2.3	-	-
USDA quality grade, %								
USDA Choice or greater	80	65	66	60	61	6.4	0.03	0.02
USDA Select	12	26	27	34	32	5.5	0.02	0.01
No USDA grade assigned[3]	8	9	7	6	7	5.5	-	-
Liver abscess, %	3.3	1.8	3.3	3.3	5.4	2.52	-	-

[1]Contrasts protected by an overall F-test $P < 0.10$

[2] 400 = Small 00; 500 = Modest 00

[3]No USDA quality grade assigned due to inferior marbling score or maturity.

CHAPTER 4 - Effects of crude glycerin on performance of growing heifers

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Abstract

A trial was conducted to evaluate effects of crude glycerin on growth performance and apparent total tract diet digestibilities of growing heifers. Crossbred heifers (n=375; 234 ± 3.2 kg BW) were used in a randomized complete block design with 3 treatments. Treatments consisted of 0, 4, or 8% crude glycerin added to forage-based growing diets. Added glycerin, along with soybean meal replaced wet corn gluten feed. All diets contained 60% corn silage and provided 300 mg monensin per heifer daily. Cattle were stratified by body weight and randomly assigned within strata to 48 partially covered, concrete-surfaced pens. Each pen contained 7 to 8 animals and there were 16 pens per treatment. Cattle had *ad libitum* access to feed and water and were fed once daily for 90 d. Apparent total tract digestibilities were calculated from total fecal collections obtained from concrete surfaced pens over a 72 or 96-h period. Feed efficiency improved by 4 and 5% when glycerin was added at 4 and 8% of the diet, respectively (linear, $P = 0.01$). Final BW, ADG, and DMI over the entire feeding period were similar ($P > 0.2$) for growing heifers fed 0, 4, or 8% glycerin. During the period where total tract digestibility was measured, glycerin decreased DM, OM, and NDF intake linearly ($P < 0.01$), but fecal outputs of DM, OM, and NDF were similar ($P > 0.76$) among treatments. Apparent total tract digestibilities of DM, OM, and NDF decreased linearly ($P < 0.01$) with increasing levels of glycerin in the diet. Feeding glycerin decreased apparent total tract diet digestibilities of DM, OM, and NDF, but improved feed efficiency.

Key Words: glycerin, digestibility, growing cattle

Introduction

Expansion of the biodiesel industry has produced surpluses of crude glycerin causing price of this byproduct to plummet (Niles, 2006). Low prices have attracted interest in crude glycerin as a source of energy for livestock. However, effects of crude glycerin on growth performance of cattle have not been studied extensively, particularly with high forage diets. Previous research by Parsons et al. (2009) evaluated effects of glycerin ranging from 0 to 16% of flaked corn finishing diets, and found that optimal growth performance was achieved by adding 2% glycerin to the diet. In contrast, Mach et al. (2009) replaced 0, 4, 8, or 12% barley grain with crude glycerin in high-concentrate diets fed to Holstein bulls and observed no differences in DMI, ADG, or G:F. Another study evaluated 0, 0.5, and 2% glycerin in finishing diets fed to feedlot heifers and found that feeding low levels of glycerin yielded no improvements in growth and decreased DMI (Schneider et al., 2010).

Some research suggests that glycerin may affect fiber digestion in ruminants. Parsons and Drouillard (2010) evaluated effects of glycerin on apparent total tract digestibilities of NDF in animals consuming high-concentrate diets and found NDF digestibilities tended to decrease linearly with increasing levels of crude glycerin. Roger et al. (1992) found that glycerol inhibited in vitro degradation of cellulose by ruminal fungi when glycerol was added at 0.5% of the media and inhibited cellulolytic bacteria when glycerol concentrations were 5% of the media. Fungi do not play a vital role in ruminal fermentation of high-concentrate diets, but suppression of cellulolytic activity by glycerin may alter performance and reduce total tract digestibilities when diets are high in fiber. The objective of this research was to evaluate effects of crude glycerin on performance and apparent total tract digestibilities of DM, OM, and NDF in growing cattle fed high forage-diets.

Materials and Methods

Growing Study

Care and handling of animals in this experiment were approved by the Kansas State University Institutional Animal Care and Use Committee. Crossbred heifers (n=375; 234 ± 3.2 kg BW) were used in a randomized complete block experiment. Incoming cattle were offered *ad libitum* access to ground alfalfa hay and water. Within 24 h of arrival and 30 d prior to the start of the study, heifers were identified with an individual ear tag, individually weighed, vaccinated with Bovishield-IV and Fortress-7 (Pfizer Animal Health, Exton, PA), injected with Micotil (Elanco, Greenfield, IN), and orally drenched with Safe-Guard (Intervet, Inc.). Upon initiation of the study, cattle were individually weighed, implanted with Synovex H (Fort Dodge Animal Health, Overland Park, KS), and randomly assigned to 1 of 48 pens. Each pen contained 7 to 8 animals, and there were 16 pens per treatment. Pens were concrete surfaced (36 m^2) and had overhead shade covering approximately 50% of the pen and the entire feed bunk. Feed bunks provided 3.2 linear m of bunk space, and fence line water fountains were shared between 2 adjacent feedlot pens. Cattle were given *ad libitum* access to treatment diets (Table 4-1), which were fed once daily at approximately 1300 h. Dietary treatments consisted of 0, 4, or 8% crude soy-based glycerin in high forage growing diets. Crude glycerin replaced wet corn gluten feed, and soybean meal was added to maintain similar protein levels across all treatments. All diets contained 60% corn silage, and provided 300 mg monensin (Elanco Animal Health, Greenfield, IN) per heifer daily. Samples of diet ingredients were collected weekly throughout the duration of the study for determination of DM and nutrient content. Crude glycerin and corn silage were analyzed by a commercial laboratory (SDK laboratories, Hutchinson, KS). Analyses performed on crude glycerin were as follows: glycerol content by HPLC according to official method

982.22 (AOAC, 1995); Moisture by Karl Fischer according to official method 966.2 (AOAC, 1995); Ash using official method 942.05 (AOAC, 1995); and methanol using official method 973.23 (AOAC, 1995). Crude glycerin contained 81.5% glycerol, 13.3% water, 6.3% ash, and less than 0.02% methanol. Corn silage utilized during the study was analyzed for glycerol content by HPLC according to official method 982.22 (AOAC, 1995) and found to contain less than 0.1% glycerol on a DM basis. Weight of each pen of heifers was determined at the beginning of the study, and on d 28, 56, and 90.

Apparent Total Tract Digestibility

Apparent total tract digestibilities of DM, OM, and NDF were determined for 46 of the 48 pens in 2 periods according to procedures described by Löest et al. (2001). Briefly, on d 46 of the feeding period, heifers from half of the treatment pens were removed from their pens at 0800 h, pen surfaces were cleaned thoroughly, and all unconsumed feed was removed from the feed bunks. Heifers were returned to their pens and fed. After 24, 48, 72, and 96 h, all feces were collected from each pen, weighed, and a representative sample was retained and immediately placed into a drying oven at 55°C. Once dried, daily samples from each pen were composited and thoroughly homogenized. Feed refusals also were collected for each of the sampling days. Digestibilities for the second half of the pens were measured in a second period beginning on d 52. Due to heavy rainfall, collections in period 2 were limited to 3 d. Digestibility measurements from 2 pens were excluded because animals from 2 pens were commingled during 1 d of the fecal collection period. Diet and fecal samples from both periods were subsequently analyzed for DM, OM, and NDF content. Dry matter and OM analyses were performed by drying a portion of the partially dried samples in a forced-air oven set to 105°C for 24 h, and samples subsequently were placed into a muffle oven for 8 h at 450°C to determine ash content. Total DM was

calculated by multiplying 105°C DM value by the 55°C value from the original sample, and total OM was calculated by subtracting the percentage of ash remaining from 100. Analyses of NDF were performed using an ANKOM fiber analyzer (ANKOM Technology Corp., Fairport, NY). Apparent total tract digestibilities for DM, OM, and NDF were calculated as [1 – (fecal DM output/DMI)] × 100%, [1 – (fecal OM output/OM intake)] × 100%, and [1 – (fecal NDF output/NDF intake)] × 100%, respectively.

Statistical Analysis

Growth performance and apparent total tract digestibility data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Pen was the experimental unit, the random effect was block, and treatment was the fixed effect. Orthogonal contrasts were used to evaluate linear and quadratic effects of glycerin, and were protected by requiring an *F*-test with $P < 0.1$.

Results and Discussion

Growth Performance

Performance data are summarized in Table 4-2. Feed efficiency improved by 4 and 5% when glycerin was added at 4 and 8% of the diet, respectively (linear, $P = 0.01$). Although glycerin improved feed efficiency when the entire feeding period was examined, when considering individual intervals during the feeding period, d 0-28 was the only time period when glycerol did not improve efficiency ($P > 0.4$). This is likely due to the inability to accurately determine ADG over a short time interval due to high error rates, but could suggest that ruminal microbial populations need time to adapt before glycerin can be most effectively utilized. To our knowledge no other studies have evaluated effects of glycerin over individual time increments during the feeding period, but similar improvements in efficiency over the entire feeding period

were reported by Parsons et al. (2009) as a result of feeding glycerin in high concentrate diets. In their study, efficiency was optimized with 2% glycerin and levels beyond 8% actually resulted in poorer efficiencies when compared to controls. Mach et al. (2009) used levels of glycerin similar to those in our experiment and found that efficiencies of finishing Holstein bulls were not different among treatments.

Final BW and ADG were not affected by adding glycerin to growing diets ($P > 0.2$) over the entire feeding period. However, during the last 34 d of the feeding period glycerin increased ADG linearly ($P < 0.01$) which may also suggest cattle may need time to adapt to glycerin or that cattle were compensating for previously poorer gains. Our data for final BW and ADG are accordance with the findings of Mach et al. (2009), who utilized 0, 4, 8, or 12% glycerin as a component of finishing diets fed to Holstein bulls, and observed no differences in final BW or ADG. In contrast, Parsons et al. (2009) observed adding glycerin at 4 or 8% to finishing diets increased final BW and ADG. Contradictions existing in the early literature might be attributable to diet differences between studies since little is known about possible interactions between glycerin and other components of the diet. Similar discrepancies exist in the scientific literature pertaining to effects of glycerin on pig performance. Lammers et al. (2008) found no differences in ADG or G:F when glycerin was added at 5 or 10% of the diet at any phase of pig production. In contrast, Groesbeck et al. (2008) fed glycerol at 0, 3, and 6% to nursery pigs and observed linear improvements in ADG as glycerin concentration of the diet was increased. Duttlinger et al. (2008) found no effects on ADG or G:F of finishing pigs when glycerin was fed at 2.5 or 5%, but another study completed by Duttlinger et al. (2009) found that 5% glycerin tended to improve G:F.

Glycerol tended to decreased DMI linearly over d 0-28 ($P = 0.1$ for overall F- test; $P < 0.05$) and decreased DMI d 28-56 ($P < 0.02$), but they became more similar over the final 34 d. As a result, DMI were similar ($P = 0.16$) over the entire feeding period. Research where glycerin was included in finishing cattle diets also suggests glycerin may decrease DMI (Parsons et al., 2009; Schneider et al., 2010).

Apparent Total Tract Digestibility

Apparent total tract digestibilities for growing heifers are summarized in Table 4-3. Glycerin decreased DM, OM, and NDF intake linearly ($P < 0.01$). This is consistent with linear decreases in DMI observed from d 29-56 because digestibility measurements were obtained from d 46-56. Similar observations have been reported in finishing studies where DMI decreased linearly as glycerin concentrations in the diet were increased (Parsons et al., 2009; Schneider et al., 2010). Fecal outputs of DM, OM, and NDF were similar ($P > 0.76$) among treatments. Apparent total tract digestibilities of DM, OM, and NDF decreased linearly ($P < 0.01$) as a result of adding glycerin to the diet. Decreases in DM and OM digestibility can be attributed largely to decreases in apparent total tract digestibility of NDF Parsons and Drouillard (2010) reported similar decreases in NDF digestibility as a result of adding glycerin to finishing diets, but found no differences in digestibilities of DM or OM. These differences are likely explained by dietary differences since NDF made up only a small portion of the diet fed by Parsons and Drouillard (2010), and therefore it had little impact on the total DM and OM digestibilities. Several *in vitro* studies also have suggested that glycerin may have deleterious effects on fiber digestion. Roger et al. (1992) found glycerol to inhibit in vitro degradation of cellulose by rumen fungi and cellulolytic bacteria when added to culture media. Likewise, Paggi et al. (2004) evaluated effects

of glycerin on IVDMD of oat hay and carboxymethyl-cellulose, and noted that glycerin decreased IVDMD of both substrates.

Although glycerin decreased apparent total tract digestibilities of DM, OM, and NDF, no negative performance effects were observed and F:G increased. Decreases in digestibility in light of improved efficiency might be explained by a potential shift in VFA profiles which may have led to a more favorable energy status. Several researchers have suggested glycerin is fermented primarily to propionate by ruminal microbes (Rémond et al., 1993; Bergner et al., 1995; Kijora et al., 1998). Possible increases in propionate production could lead to more efficient energy utilization. Also, the magnitude decrease in NDF digested in glycerin diets is larger than the magnitude decrease in OM digestibility, therefore digestibility of other dietary components may have increased.

Addition of glycerin to high-forage growing diets improved feed efficiency but did not affect ADG or final BW. This is likely due to the portion of the feeding period when glycerol decreased DMI compared to control animals, while ADG remained similar across treatment groups. Though not significant, the trend toward improved efficiency of glycerin fed cattle later in the study may imply that there is some adaption to glycerin over time, or that cattle in those treatment groups were simply compensating for previously poorer gains. Although ADG were similar, it is possible that total energy deposition was different among diets, which could also affect efficiency. Understanding the effects of crude glycerin on rumen fermentation and possible interaction between glycerin and other dietary components will be necessary to determine the relative feeding value of glycerin in high forage growing diets.

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Table 4-1. Composition of diets containing 0, 4, or 8% crude glycerin fed to growing heifers

Item	0% Glycerin	4% Glycerin	8% Glycerin
Ingredients, % of DM			
Corn silage	60.0	60.0	60.0
Wet corn gluten feed	35.0	30.2	25.4
Crude glycerin	-	4.0	8.0
Soybean meal	-	0.8	1.6
Limestone	1.6	1.6	1.6
Urea	0.4	0.4	0.4
Vitamin/mineral premix[1]	0.3	0.3	0.3
Feed additive premix[2]	2.7	2.7	2.7
Analyzed composition, %			
DM	43.3	43.7	44.2
OM	93.4	93.2	93.7
CP	13.0	12.5	12.1
NDF	36.2	34.7	33.2
Calcium	0.9	0.9	0.9

[1]Formulated to provide 0.1 mg Co, 10 mg of Cu, 0.6 mg of I, 60 mg of Mn, 0.25 mg Se, 60 mg Zn, and 2200 IU vitamin A, per kg of diet DM.
[2]Feed additive premix provided 300 mg of monensin (Elanco Animal Health, Greenfield, IN) per animal daily in a ground corn carrier.

Table 4-2. Performance for growing heifers fed high roughage diets containing 0, 4, or 8% crude glycerin

	Dietary Glycerin, %				*P* – values		
Item	0%	4%	8%	SEM	*F*-Test	Linear	Quadratic
No. of pens (heifers)	16 (124)	16 (125)	16 (126)	-	-	-	-
Days on feed	90	90	90	-	-	-	-
Initial weight, kg	234	234	234	3.2	0.99	0.91	0.98
Final weight, kg	368	370	369	4.0	0.95	0.84	0.82
Dry matter intake, kg/day							
d 0-28	7.21	6.94	6.80	0.17	0.14	0.05	0.71
d 29-56	9.62	9.35	9.13	0.14	0.07	0.02	0.91
d 57-90	9.64	9.39	9.45	0.14	0.40	0.32	0.37
d 0-90	8.88	8.62	8.53	0.14	0.16	0.06	0.60
Average daily gain, kg/day							
d 0-28	1.89	1.80	1.73	0.056	0.14	0.05	0.90
d 29-56	1.75	1.79	1.76	0.031	0.65	0.82	0.37
d 57-90	0.95	1.02	1.09	0.036	0.03	< 0.01	0.94
d 0-90	1.49	1.50	1.50	0.023	0.93	0.84	0.74
G:F, kg/kg							
d 0-28	0.264	0.260	0.255	0.0076	0.72	0.43	0.90
d 29-56	0.182	0.192	0.193	0.0034	0.05	0.03	0.29
d 57-90	0.098	0.109	0.115	0.0035	< 0.01	< 0.01	0.65
d 0-90	0.168	0.175	0.176	0.0020	< 0.01	< 0.01	0.21

Table 4-3. Apparent total tract digestibilities for growing heifers fed high roughage diets containing 0, 4, or 8% crude glycerin

	Dietary glycerin, %				Contrast *P* – values		
Item	0%	4%	8%	SEM	*F* - Test	Linear	Quadratic
Intake, kg/d							
DM	9.51	8.84	8.49	0.17	< 0.01	< 0.01	0.41
OM	8.87	8.24	7.97	0.15	< 0.01	< 0.01	0.32
NDF	3.60	3.21	2.96	0.06	< 0.01	< 0.01	0.35
Fecal output, kg/d							
DM	2.94	2.92	2.87	0.07	0.76	0.49	0.86
OM	2.43	2.42	2.39	0.06	0.82	0.56	0.84
NDF	1.50	1.48	1.46	0.05	0.79	0.50	0.92
Amount digested, kg/d							
DM	6.58	5.92	5.62	0.14	< 0.01	< 0.01	0.24
OM	6.44	5.82	5.59	0.13	< 0.01	< 0.01	0.19
NDF	2.10	1.73	1.49	0.06	< 0.01	< 0.01	0.35
Apparent total tract digestibility, %							
DM	68.9	67.0	66.1	0.62	< 0.01	< 0.01	0.37
OM	72.4	70.5	70.0	0.64	< 0.01	< 0.01	0.29
NDF	58.4	53.8	50.3	1.38	< 0.01	< 0.01	0.73

CHAPTER 5 - Glycerol as a solvent in non-enzymatic browning processes intended to improve ruminal bypass characteristics of protein-rich feedstuffs

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Abstract

Several experiments were conducted to determine if glycerol could serve as a replacement for water as a solvent in processes designed to facilitate non-enzymatic browning (NEB) of protein meals. In experiment 1, soybean meal was combined with 8% glycerol; 6% glycerol with 2% water; 4% glycerol with 4% water; 2% glycerol with 6% water; and 8% water. Xylose was added as a reducing sugar, or, alternatively, the mixtures were pretreated with carbohydrase enzymes or yeast to generate reducing sugars from carbohydrates in soybean meal. Initial weights were obtained, products were processed, and weights were recorded again to evaluate moisture losses associated with evaporation. Energy losses were calculated from resulting moisture losses during processing for each respective product. Experiment 2 monitored temperature change in mixtures of soybean meal and 4 solvent treatments consisting of different combinations of glycerin and water (9% glycerin; 6% glycerin with 3% water; 3% glycerin with 6% water; 9% water) for each previously described method of providing reducing sugars. Products were heated and core temperatures were recorded for the duration of the heating process to evaluate effects of glycerol on process temperatures. Experiment 3 utilized products manufactured in experiment 1 to evaluate effects of using glycerol as a solvent in NEB processes on resistance of protein to degradation by ruminal microbes in an *in vitro* ammonia release assay. In experiment 4, soybean meal that was browned with 10% glycerol and yeast was fed to 58 crossbred heifers as a component of a corn-based finishing diet. The performance and carcass characteristics of these heifers were then compared to heifers fed a diet containing a similar level of glycerin and untreated soybean meal. Experiment 1 revealed that total process moisture loss decreased linearly ($P < 0.001$) in all products as more water was replaced with glycerin, and therefore glycerin decreased energy losses. In experiment 2, core temperatures increased linearly

(P < 0.001) with higher levels of glycerin in processes involving yeast and xylose as the method of introducing reducing sugars. In the process involving invertase, increasing glycerin had a linear (P = 0.001) and quadratic (P = 0.02) effect on core temperatures throughout the process. All processing methods in experiment 3, regardless of solvent treatment, decreased nitrogen degradation compared to the untreated control soybean meal ($P < 0.01$). Increasing glycerin in test products made with yeast yielded linear decreases in nitrogen degradation ($P < 0.01$). Glycerin level had a linear ($P < 0.01$) and a quadratic effect ($P < 0.01$) on nitrogen degradation of test products processed with the invertase method. In contrast, there were no significant linear or quadratic effects of glycerin on nitrogen degradation of products made using xylose. In experiment 4, no differences in performance or carcass characteristics were observed as a result of adding glycerol as NEB soybean meal when compared to the direct addition of glycerol to the diet at a similar level. Glycerol, when added as a component of NEB soybean meal decreased ADG and Final body when compared to the control diet. Performance data were similar for cattle receiving glycerin as a direct addition to the diet and cattle receiving no glycerin. Glycerol may serve as a more suitable solvent for NEB processes than water because its chemical and physical properties can enhance browning processes while increasing process efficiency, yielding products from some processes with superior resistance to microbial degradation.

Introduction

Soybean meal can be heated in the presence of reducing sugars to promote a non-enzymatic browning (Maillard) reaction, creating value-added products that contain proteins that resist degradation in the rumen (Cleale et al., 1987a), and in some applications, results in more efficient protein utilization by ruminants (Cleale et al., 1987b; Nakamura et al.,1992). For these

reasons, the Maillard reaction has been exploited commercially to produce protein meals with improved nutritional characteristics for ruminant animals.

Several methods have been developed to provide reducing sugars for a non-enzymatic browning (NEB) reaction and generally can be divided into 3 separate processes: 1. direct addition of the reducing sugars, usually xylose because it is the most reactive (Cleale et al., 1987a) or other commercial sources of xylose such as sulfite liquor (Nakamura et al., 1992); 2. addition of a carbohydrase enzyme which under the proper conditions will convert sugars within soybean meal to reducing forms (Coetzer, 2000); 3. substitution of the enzyme with an organism, such as *Saccharomyces cerevisiae* that is capable of producing a carbohydrase enzyme such as (Drouillard and Coetzer, 2003). For the purposes of this article, methods 1, 2, and 3 will be defined as the xylose, invertase, and yeast methods. All of the previously described methods of providing the reducing sugar have been implemented in production processes where water is utilized as a solvent. We hypothesized that glycerin may serve as a more economical solvent than water in these processes by decreasing energy inputs due to the higher vaporization point (thus less evaporative heat loss) of glycerol. Furthermore, glycerol may further enhance the browning process. Addition of glycerol to food model formulations containing sodium caseinate and glucose has been shown to increase rates of naturally occurring Maillard reactions as much as 1.5 fold compared to control formulations without glycerol, suggesting it may operate as a solvent (Sherwin and Labuza, 2003). Similarly, Mustapha and co-workers (1998) found that although the reactants were not entirely soluble in glycerol, more extensive browning of lysine and xylose mixtures occurred in glycerol than in an aqueous solution. Other research suggests that glycerol may serve as a precursor in the Maillard reaction (Cherny and Guntz, 2006), and heating a

mixture of glycerol and amino acids in the absence of reducing sugars will result in a certain degree of browning (Obanu et al., 1977).

The presence of glycerol in non-enzymatically browned soybean meal may also have positive implications for animal performance. Parsons et al. (2009) reported that adding low levels of glycerin to the diet increased feed efficiency and ADG of feedlot heifers. Objectives of our research were to 1) identify effects of replacing water with glycerol or combinations of glycerol and water in non-enzymatic browning processes on process efficiency, extent of browning, and resistance to microbial degradation of browned soybean meal products and 2) evaluate effects of feeding soybean meal that is browned using glycerol as a solvent on performance and carcass characteristics of feedlot cattle.

Materials and Methods

Care and handling of animals used in the following experiments were performed under approval of the Kansas State University Institutional Animal Care and Use Committee.

Experiment 1

Dehulled soybean meal was used to manufacture browned soybean meal in a 4 × 5 factorial design to monitor effects of solvent and reagent on product moisture loss due to evaporation during processing. Soybean meal was combined on a wt/wt basis with one of 5 solvent treatments and 1 of 4 reagents in 150-g batches. Solvents consisted of varying proportions of technical grade glycerol (99% pure glycerol) and water at the following concentrations: 8% glycerol, 0% water; 6% glycerol, 2% water; 4% glycerol, 4% water; 2% glycerol, 6% water; 0% glycerol and 8% water. Reagents included no reagent or the addition of 2% xylose (99% pure, Sigma-Aldrich, St. Louis, MO), 0.1% invertase (10,000 units/g, reagent

grade; Carolina Biological, Burlington, NC), or 1% dry baker's yeast (AB Mauri Fleischmann's, Chesterfield, MO). Products were hand mixed in individual batches for 1 min and then placed into 20 × 14 × 3 cm aluminum pans, weighed, and covered with aluminum foil. For products containing xylose or no reagent, pans were placed directly into a preheated forced-air oven set at 150°C for 60 min. Products containing invertase and yeast were first allowed to steep in a convection oven for 60 minutes set at 50°C (invertase products) or 30°C (yeast products), and then heated in a forced-air oven at 150°C for 60 min. After heating, aluminum foil was removed and products were dried in convection oven at 45°C for 48 h to remove added moisture and prevent molding. Following drying, products were allowed to air equilibrate for 12 h and weighed. Product moisture loss was calculated for each individual product and expressed as a percentage of moisture lost during processing, and energy loss was determined by multiplying the amount of energy required to evaporate one g of water by the total number of g evaporated for each product. Each product was manufactured on each of 3 d.

Data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC) with individual product as the experimental unit, day as the random effect, and solvent, reagent, and solvent × reagent as fixed effects. Orthogonal contrasts were used to evaluate linear and quadratic effects of glycerol level within solvent.

Experiment 2

Soybean meal was combined with xylose and 4 solvent treatments consisting of different combinations of glycerin and water (9% glycerin, 6% glycerin with 3% water, 3% glycerin with 6% water, or 9% water) to evaluate effects of solvent on temperature change during processing. On 3 consecutive d, all test products were mixed according to procedures described for experiment 1. Individual products were transferred to two 30 x 200 mm test tubes, covered with

rubber stoppers, and a temperature probe was suspended in the center of the product. The tubes were then placed in a random slot of a test tube rack and the rack was placed in a preheated forced air oven at 150°C. Temperature probes were attached to a XR5 data logger (Pace Scientific Inc, Mooresville, NC),and temperatures were recorded at 10 s intervals for 70 min. Average temperatures for each minute of the heating process were calculated from temperature readings. The previously described process was repeated for the invertase and yeast methods with the addition of the steeping step as described in experiment 1.

Data for each method of providing reducing sugars were analyzed by repeated measures analysis using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The model statement included treatment, time, and treatment × time, with individual product as the experimental unit, and day as the random effect. Treatment means for each min were determined using the LSMEANS option. Linear and quadratic effects of glycerol level within solvent were evaluated using orthogonal contrasts.

Experiment 3

Susceptibility of protein to degradation by ruminal microbes was evaluated for NEB soybean meal produced in experiment 1. Xylose, invertase, and yeast products were utilized in separate *in vitro* ammonia release assays. Whole rumen contents were obtained from a ruminally cannulated steer fed a high roughage diet consisting primarily of alfalfa hay. Ruminal contents were strained through 2 layers of cheese cloth and then strained again through 8 layers of cheese cloth to remove large feed particles. The inoculum was prepared so that 1 L would contain 400 mL of strained rumen fluid, 400 mL of McDougall's buffer, 50 mL of a solution containing 100 mg/mL of maltose, 25 mL of a chloramphenicol solution containing 1.8 mg/mL of chloramphenicol, 25 mL of a 60 m*M* hydrazine sulphate solution, and 234 mg of 2-

mercaptoethanol. Hydrazine sulfate were added to the inoculum to inhibit utilization of NH_3 by ruminal microbes and chloramphenicol to prevent use of amino acids for protein synthesis.

Browned soybean meal products and a control soybean meal sample that was not browned were finely ground using a cyclone mill (Udy Corporation; Fort Collins, CO) and nitrogen contents of browned soybean meal products were determined using a Leco N analyzer (Leco Corporation, St. Joseph, MI; AOAC, 1995). Ground products were weighed in duplicate for each time point into 26 x 100 mm centrifuge tubes so that each tube contained 4 mg of N. McDougall's buffer (8 mL) was added approximately 1 h prior to starting the assay. Also, blank tubes containing no substrate were included in duplicate for each of the 4 time points of 0, 4, 8, and 12 h. Tubes were then covered with aluminum foil and placed in an incubator set at 39°C to be prewarmed. All components of the inoculum were mixed just prior to starting the assay, and 20 mL of inoculum were then added to the tubes and tubes were gassed with CO_2, stoppered, and placed in a shaking water bath at 39°C. At each respective time point, incubation was terminated by adding 2 mL of 65% (w/v) trichloroacetic acid to each tube and subsequently placing them into an ice bath. Tubes were then frozen until analysis.

For analysis, samples were thawed and centrifuged at 21,000 $\times$ g for 15 min at 4°C. The supernatant was collected and analyzed for NH_3 concentration using a Technicon III AutoAnalyzer (Technicon Instruments Corporation, Tarrytown, NY). Percent nitrogen remaining in each tube after incubation was determined by converting mg NH_3 to mg of N based on the molar ratio of nitrogen to NH_3. Percent nitrogen degraded (PD) was then determined for each tube using the following formula.

$$\text{PD} = \frac{\text{mg N per tube} - \text{mg N per blank}}{\text{initial mg N per tube}} \times 100$$

Statistical analyses were performed to evaluate the 5 solvent treatments with each method of providing reducing sugar using repeated measures analysis in the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The model statement included the effects of time, treatment, and treatment by time. The experimental unit was product and the random effects were day and treatment × day. Treatment means for each time point were determined using the LSMEANS option. Orthogonal contrasts were used to evaluate linear and quadratic effects of glycerol level within the substrate.

Experiment 4

Crossbred heifers (n=177; 427 ± 9 kg) were used to evaluate effects of feeding NEB soybean meal containing glycerin to feedlot heifers to determine if possible performance benefits associated with glycerin feeding would be present if glycerol was added to the diet as a component of NEB soybean meal. Incoming cattle were allowed free access to ground alfalfa hay and were processed within 24 h of arrival. During processing, heifers were identified with an individual ear tag, individually weighed, implanted with Revalor 200 (Intervet, Inc., Millsboro, DE), vaccinated with Bovishield-IV and Fortress-7 (Pfizer Animal Health, Exton, PA), injected with Micotil (Elanco, Greenfield, IN), and drenched with Safe-Guard (Intervet, Inc.) for internal parasites. Four weeks after initial processing, cattle were revaccinated with Bovishield-IV. Prior to initiating finishing treatments, cattle were fed a series of step-up rations to gradually adapt them to their final finishing rations. At the end of the step-up phase, cattle were stratified by body weight and randomly assigned (within strata) to 24 pens containing 7 to 8 animals per pen, with 8 pens per treatment. Pens were concrete surfaced (36 m^2) and had overhead shade covering one-half of the pen and the entire feed bunk. Feed bunks provided 3.2 linear m of bunk space, and fence line water fountains were shared between 2 adjacent feedlot pens. Cattle were

given ad libitum access to treatment diets, which were fed once daily at approximately 1100 h. Experimental treatment diets (Table 5-1) were based on corn and consisted of 0 (SBM) or 0.5% crude soy-based glycerin as a direct addition to the gain-based diets (GSBM) or 0.5% crude glycerin as a component of NEB soybean meal (NEBGSBM). The GSBM diet contained an equivalent amount of untreated soybean meal along with 0.5% glycerin to serve as a direct addition to the diet (unprocessed) to serve as a direct comparison to the NEBSBM diet. Crude glycerin was analyzed by a commercial laboratory (SDK laboratories, Hutchinson, KS) as follows: moisture by Karl Fischer titration according to official method 966.2 (AOAC, 1995); ash using official method 942.05 (AOAC, 1995); Na using official method 956.01 (AOAC, 1995); N using official method 920.176 (AOAC, 1995); and methanol using official method 973.23 (AOAC, 1995). Glycerin contained 14.3% moisture, 6.68% ash, 2.58% Na, 0.04% N, and less than 0.01% methanol. Diets were based on dry-rolled corn for the first 37 d of the feeding period, then gradually transitioned to diets based on steam-flaked corn. All diets contained 3% alfalfa hay and 6% corn silage, and provided 300 mg monensin, 90 mg tylosin, and 0.5 mg melengestrol acetate (Elanco Animal Health, Greenfield, IN) per heifer daily. Heifers were also fed zilpaterol HCl (Intervet Inc., Millsboro, DE) at a level of 8.33 mg/kg for 21 d with a 3-d withdrawal.

Non-enzymatically browned soybean meal for this study was manufactured in 81.6 kg batches by mixing soybean meal with 10% crude glycerin and 1% active dry yeast (Lesaffre Yeast Corporation, Milwaukee, WI) in a ribbon mixer (Davis, Bonner Springs, KS) for 3 min. The mixture was weighed into 37.9 L roasting pans, with each containing 6.8 kg of product. The two-step browning process was completed in 2 forced-air ovens consisted of a steeping period at 55°C for 1 h followed by a 3-h heating period at 150°C. Contents of each pan were re-mixed

half-way through the tempering step and hourly throughout the high temperature heating period to facilitate uniform browning. A glycerol level of 10% was chosen for production of NEB soybean meal so that NEB soybean meal would contribute 0.5% glycerin to the total diet.

Weight of each pen of heifers was determined at the beginning of the experiment and immediately prior to slaughter. After 89 d on feed, cattle were transported to a commercial abattoir in Holcomb, KS and harvested. Hot carcass weights and incidence and severity of liver abscesses were recorded on the day of harvest. USDA quality grade; USDA yield grade; marbling score; 12th rib fat thickness; LM area; and kidney, pelvic, and heart fat were recorded after a 48-h period of chilling

Performance and carcass characteristics were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC). Pen was the experimental unit, random effect was block, and treatment was the fixed effect. Percentages of USDA yield grade, USDA quality grade, and liver abscesses were calculated and analyzed using the MIXED procedure of SAS with pen as the experimental unit, block as the random effect, and diet as the fixed effect. Orthogonal contrasts were used to evaluate effects of NEBSBM by comparing it to UTSBM. Orthogonal contrasts and pair-wise comparisons were protected by requiring an overall F – test with $P < 0.1$.

Results and Discussion

Experiment 1

No interactions were found between solvent type and reagent for effects on moisture loss, and the main effect of method of providing the reducing sugar was not significant. As a result, only effects of solvent on moisture loss are presented (Figure 5-1). Energy required to produce moisture losses in each product is expressed as energy lost during processing in Figure 5-2. Total

process moisture loss decreased linearly in all products as more water was replaced with glycerin ($P < 0.001$). Differences likely are due to evaporation of water contained within the products. Therefore, these results were expected as products prepared with glycerin contained less water and would be expected to have less evaporative losses due to the low moisture content of glycerin and the high vaporization point of glycerol compared to water. However, these results remain important because they measure water loss due to evaporation. This loss can be directly correlated to a loss in energy during processing because the amount of latent heat needed to produce decreases in product moisture content represents an energy loss, as none of this energy contributes the browning process. Products manufactured using 8% glycerin instead of 8% water resulted in a 23.7 kJ reduction in the amount of energy lost during processing. Reduced evaporative losses in glycerin products decreased thermal energy requirements for processing as evaporative heat losses were minimized. As a result, glycerin reduced energy loss in the production system.

Experiment 2

Product temperatures during processing are shown in Figures 5.2 to 5.4. Core temperatures increased linearly ($P < 0.001$) with higher levels of glycerin in yeast and xylose processes. In the process involving invertase, increasing glycerin had linear ($P = 0.001$) and quadratic ($P = 0.02$) effects on core temperatures throughout processing. Some of the temperature curves suggest products containing 9% water heat more rapidly than any of the other products until they reach 100°C. This is likely due to the higher thermal conductivity (ability to transfer heat) of water compared to glycerol. The increase in the rate of heating stopped at 100°C because water had reached its vaporization point. At this point the higher vaporization of glycerol allowed temperatures of products containing glycerol to continue to rise whereas

products containing water stopped heating and remained at approximately 100°C for the rest of the process, resulting in loss of heat due to evaporation. This is in accordance with decreases in evaporative losses and energy losses during processing as result of adding glycerol observed in experiment 1, which suggests a decrease in thermal energy requirements for processing products containing glycerin.

Experiment 3

In vitro ammonia release data expressed as a percentage of nitrogen degraded are shown in Figures 5.5 to 5.7. Main effects of treatment, time, and treatment by time were significant for all assays ($P < 0.01$). All processing methods, regardless of solvent treatment, caused decreases in nitrogen degradation compared to the control soybean meal ($P < 0.01$). Increasing glycerin in yeast products yielded linear decreases in nitrogen degradation ($P < 0.01$). Glycerin level had linear ($P < 0.01$) and a quadratic effects ($P < 0.01$) on nitrogen degradation of test products processed with the invertase method. Therefore, using glycerol in place of water in the NEB process with invertase and yeast led to products with greater resistance to microbial degradation. Because resistance to protein degradation by ruminal microbes is related to extent of browning (Cleale et al., 1987a), these findings are in agreement with the findings of researchers that reported an increase in the extent of browning when glycerol was added to intermediate moisture model systems (Cherny and Guntz, 2006; Mustapha et al., 1998; Sherwin and Labuza, 2003). In contrast, there were no significant linear or quadratic effects of glycerin on nitrogen degradation of products made using xylose. This might be attributed to greater rates of NEB reactions when adding xylose compared to the other methods used for providing reducing sugars. Cleale et al. (1987a) listed xylose as the most reactive reducing sugar in NEB reactions, yielding more advanced Maillard products than other reducing sugars. Potential benefits of adding glycerin

may not be realized when using the xylose method because browning sufficient to prevent microbial degradation may be achieved early in the process before energy losses can occur, which is likely when water reaches its vaporization point. Although the results of the assays for the xylose products did not yield clear linear effects of glycerol, the main effect of treatment was significant and some treatment differences were identified at certain time points using pair-wise comparisons. Solvents containing 6% glycerol and 2% water appeared to yield products with superior resistance to degradation by ruminal microbes and were significantly different from all other treatments after 12 h of incubation ($P < 0.05$). Products manufactured using 4% glycerol and 4% water appeared to yield the least resistance, differing from products containing 6% glycerol after only 4 h of incubation ($P < 0.05$) and tending ($P < 0.1$) to have less resistance to microbial degradation compared to other products after 12 h of incubation.

Experiment 4.

Performance data and carcass characteristics for cattle fed corn-based finishing diets are summarized in Table 5-2. Including glycerin either as GSBM or NEBGSBM decreased DMI ($P < 0.05$) when compared to the SBM treatment. Similarly, Parsons et al. (2009) reported a linear decrease in DMI as glycerin concentrations were increased in the diet, but DMI did not decrease until glycerin was fed at 4% of diet DM, which is much greater than the 0.5% levels evaluated in our study. Feeding NEBGSBM decreased ADG ($P = 0.03$) and final BW ($P = 0.02$) when compared to the SBM treatment, but were not different ($P > 0.3$) when compared to GSBM. Adding 0.5% glycerol as a direct addition to the diet (GSBM) did not affect final BW, ADG, or G:F ($P > 0.1$) to the SBM treatment. This is in accordance with the results of Mach et al. (2009) who utilized 0, 4, 8, or 12% glycerin as a component of finishing diets fed to Holstein bulls, and observed no differences in final BW or ADG. In contrast, Parsons et al. (2009) included glycerin

at levels ranging from 0 to 16% glycerin and observed a quadratic response with optimal efficiency occurring when glycerol was included at 2% of diet DM.

Carcass characteristics for heifers are summarized in Table 5-2. No differences were observed for HCW or LM area ($P > 0.10$) as a result of adding glycerin as GSBM or NEBGSBM. This is in contrast to the observation of Parsons et al. (2009), who reported an increase in HCW and LM area when glycerol was added at 2% of the diet DM. Though no treatment differences ($P > 0.7$) were identified for marbling score, glycerin when added as GSBM or NEBGSBM decreased ($P < 0.05$) the percentage of carcasses that graded USDA Choice or higher, and increased ($P < 0.05$) the percentage of carcasses that graded USDA Select. Parsons et al. (2009) observed decreases in USDA quality grade with tendencies for glycerin to cause linear decreases in the number of cattle grading USDA Choice and increase the percentage of carcasses grading USDA Select. No other differences in carcass characteristics were observed as a result of adding glycerol as GSBM or NEBGSBM. In contrast to previous studies, feeding glycerol yielded no positive effects on animal performance. Feeding glycerin as a component of NEB soybean meal decreased final BW and ADG compared to the SBM treatment. Although not significant due to high variability among cattle responses to glycerin, the GSBM treatment resulted in numerical decreases in final BW and ADG may actually suggest negative effects on animal performance. The direct comparison of GSBM and NEBGSBM yielded no differences in growth performance or carcass characteristics which might be expected because feedlot cattle fed finishing diets may not be the best model to evaluate *in vivo* effects of escape protein.

Substitution of glycerin for water in NEB processes can reduce total process moisture loss leading to more efficient browning. Similarly, glycerol may be effectively used to decrease

thermal energy requirements of processing further improving efficiency. Furthermore some processes involving glycerol yield protein meals with superior resistance to microbial degradation when compared to processes involving only water as a solvent. In the finishing study, the direct comparison of GSBM and NEBGSBM yielded no differences in performance or carcass characteristics. Although glycerin has been shown to have positive effects on animal performance, no such benefits were observed in this study and feeding glycerin as a component of browned soybean meal decreased ADG and final BW.

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Table 5-1 Composition of steam-flaked corn finishing diets with 0% glycerin (SBM), 0.5% glycerin as a direct addition to the diet (GSBM), or 0.5% glycerin added as browned soybean meal (NEBGSBM) fed to yearling heifers

Item	SBM	GSBM	NEBGSBM
Ingredients, % of DM			
Steam-flaked corn	80.6	80.0	80.0
Corn silage	6.0	6.0	6.0
Soybean meal	4.4	4.5	-
NEB soybean meal	-	-	5.0
Alfalfa hay	3.0	3.0	3.0
Crude glycerin	-	0.5	-
Limestone	1.7	1.7	1.7
Urea	1.2	1.2	1.2
Vitamin/mineral premix[1]	0.9	0.9	0.9
Feed additive premix[2]	2.2	2.2	2.2
Analyzed composition, %			
DM	76.1	76.2	76.2
CP	14.4	14.4	14.4
Ca	0.72	0.73	0.73
P	0.27	0.27	0.27
NDF	11.9	11.9	11.9

[1]Formulated to provide 0.1 mg Co, 10 mg of Cu, 0.6 mg of I, 60 mg of Mn, 0.25 mg Se, 60 mg Zn, 2640 IU vitamin A, and 11 IU vitamin E per kg diet DM.

[2]Feed additive premix provided 300 mg of monensin (Elanco Animal Health, Greenfield, IN), 90 mg tylosin (Elanco), and 0.5 mg of melengestrol acetate (Pfizer Animal Health, Exton, PA) per animal daily in a ground corn carrier. Also, zilpaterol HCl (Intervet Inc., Millsboro, DE) was fed for 21d before harvest at the rate of 8.33 mg/kg diet DM, followed by a 3 d withdrawal period.

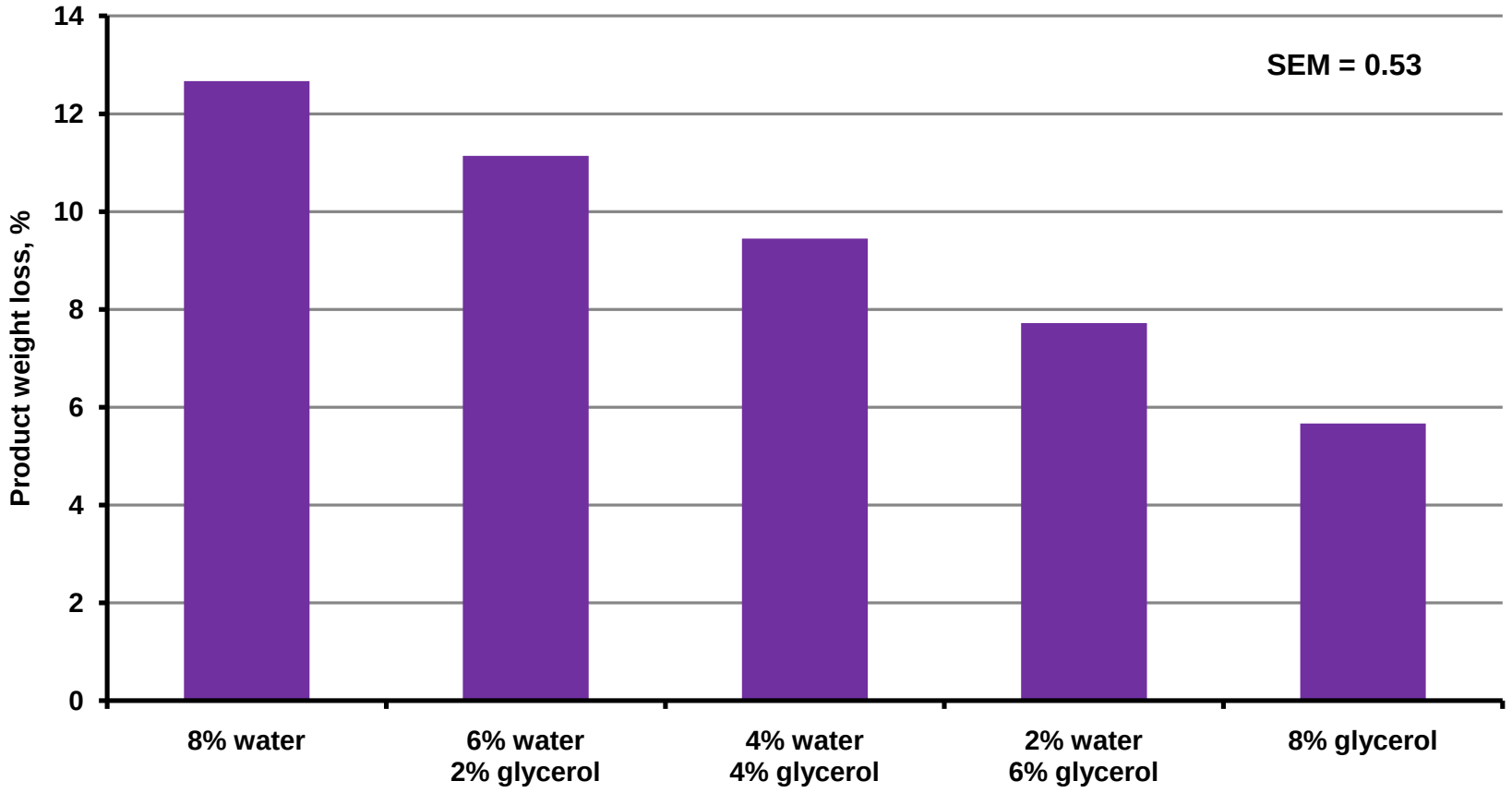
[3]NRC (2000) feed library NDF values for soybean meal were used in calculation of NDF content.

Table 5-2 Animal growth, performance, and carcass characteristics for yearling heifers fed finishing diets based on corn with 0% glycerin (SBM), 0.5% glycerin as a direct addition to the diet (GSBM), or 0.5% glycerin added as browned soybean meal (NEBGSBM)

Item	SBM	GSBM	NEBGSBM	SEM
No. of pens (heifers)	8 (61)	8 (58)	8 (58)	-
Initial BW, kg	427	427	427	9.7
Final BW, kg	547[c]	536[cd]	529[d]	10.0
DMI, kg	8.8[c]	8.5[d]	8.4[d]	0.15
ADG, kg/d	1.34[c]	1.22[cd]	1.15[cd]	0.06
G:F	0.152	0.145	0.137	0.007
Hot carcass weight, kg	357	352	343	5.55
Dressed yield, %	65.4	65.7	65.9	0.56
LM area, cm^2	91.9	91.7	89.5	0.94
12^{th} rib fat thickness, cm	1.31	1.26	1.42	0.10
KPH, %	2.1	2.0	2.0	0.1
Marbling[a]	450	430	425	13.4
USDA yield grade (YG),	2.1	2.0	2.2	0.14
YG 1, %	24	21	21	5.6
YG 2, %	45	50	41	7.3
YG 3, %	29	27	33	7.9
YG 4, %	2	2	5	2.1
USDA quality grade, %				
USDA premium Choice	26	16	14	5.1
USDA Choice or greater	80[c]	65[d]	58[d]	6.6
USDA Select	12[c]	26[d]	33[d]	4.9
No USDA grade assigned[b]	8	9	9	5.8
Liver abscess, %	3.3	1.8	3.1	2.4

[a] 400 = Small 00; 500 = Modest 00
[b]No USDA grade assigned due to inferior marbling score or maturity
[c,d]Means without a like superscript are different ($P < 0.05$)

Figure 5-1 Moisture loss during processing for NEB soybean meal products containing various combinations of glycerol and water browned with xylose, invertase, yeast or no method of providing a reducing sugar[a]



[a]Effect of glycerol (Linear, $P < 0.001$)

Figure 5-2 Energy loss during processing due to evaporation for NEB soybean meal products containing various combinations of glycerol and water browned with xylose, invertase, yeast or no method of providing a reducing sugar

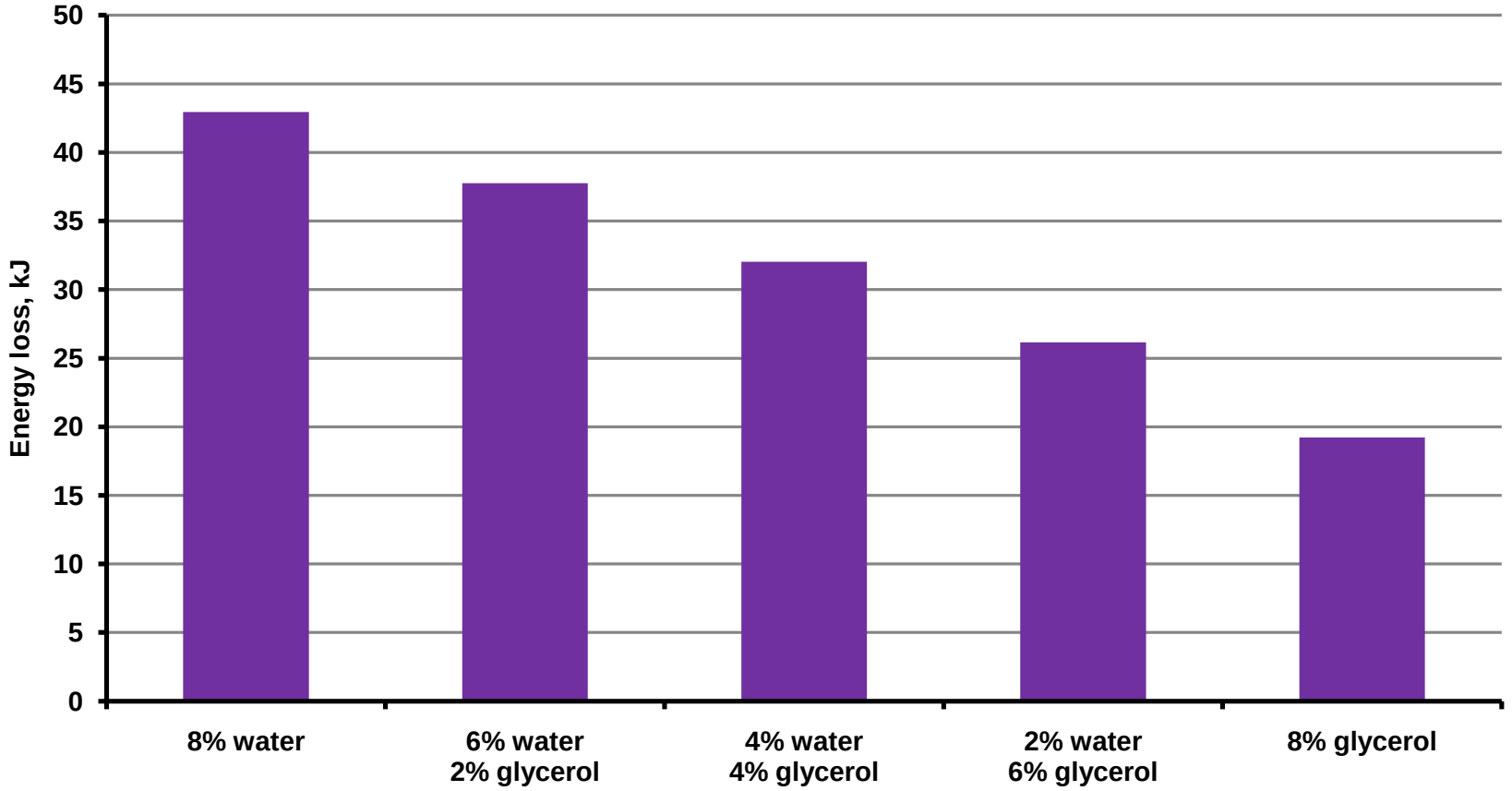
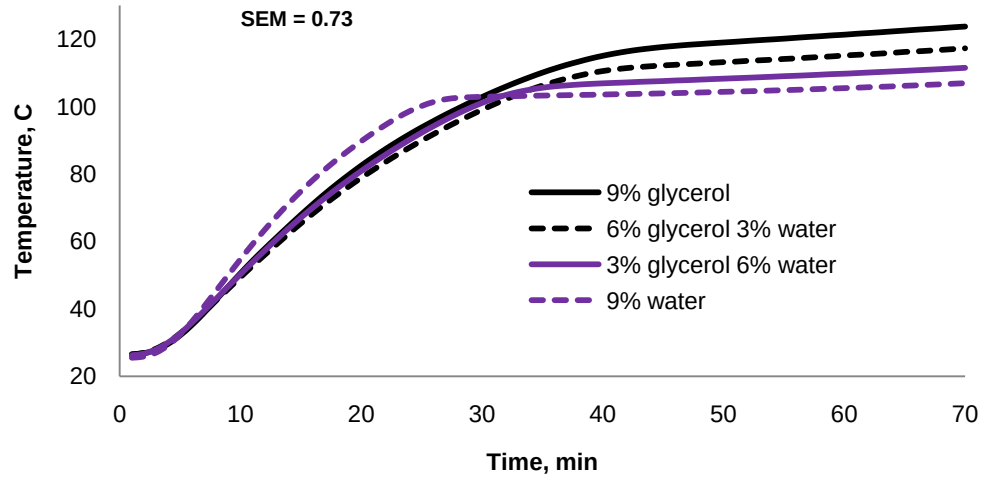
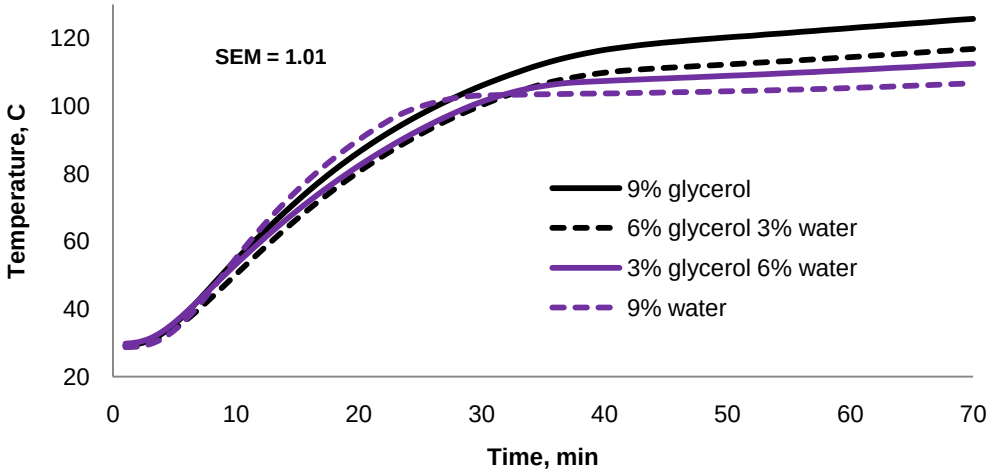


Figure 5-3 Changes in product temperature during processing with different solvent combinations in NEB processes utilizing xylose[a]



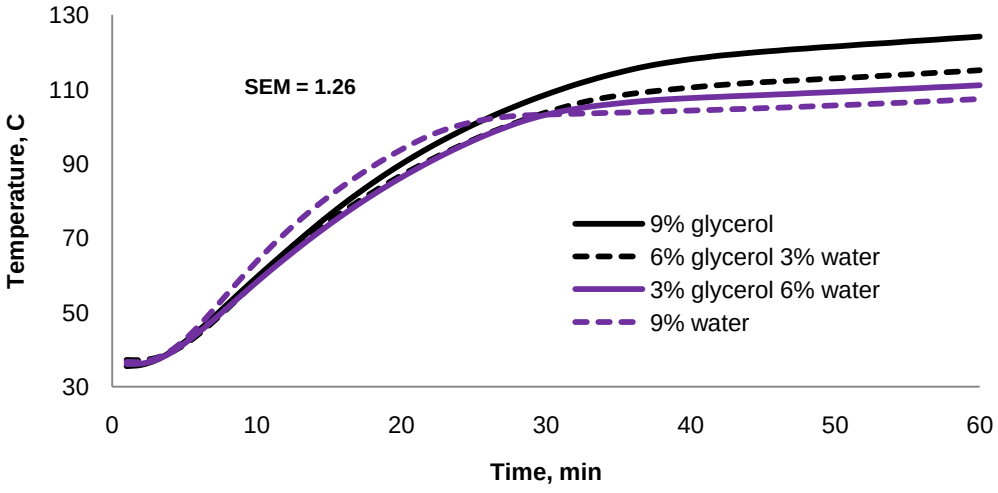
[a]Effect of glycerol (Linear, P <0.01)

Figure 5-4 Changes in product temperature during processing with different solvent combinations in NEB processes utilizing yeast[a]



[a]Effect of glycerol (Linear, P <0.01)

Figure 5-5 Changes in product temperature during processing with different solvent combinations in NEB processes utilizing invertase[a]



[a]Effect of glycerol (Linear, P <0.01; Quadratic, P <0.01)

Figure 5-6. Effect of solvent on *in vitro* N degradation by ruminal microflora during a 12 h incubation period of soybean meal (SBM) products browned using xylose

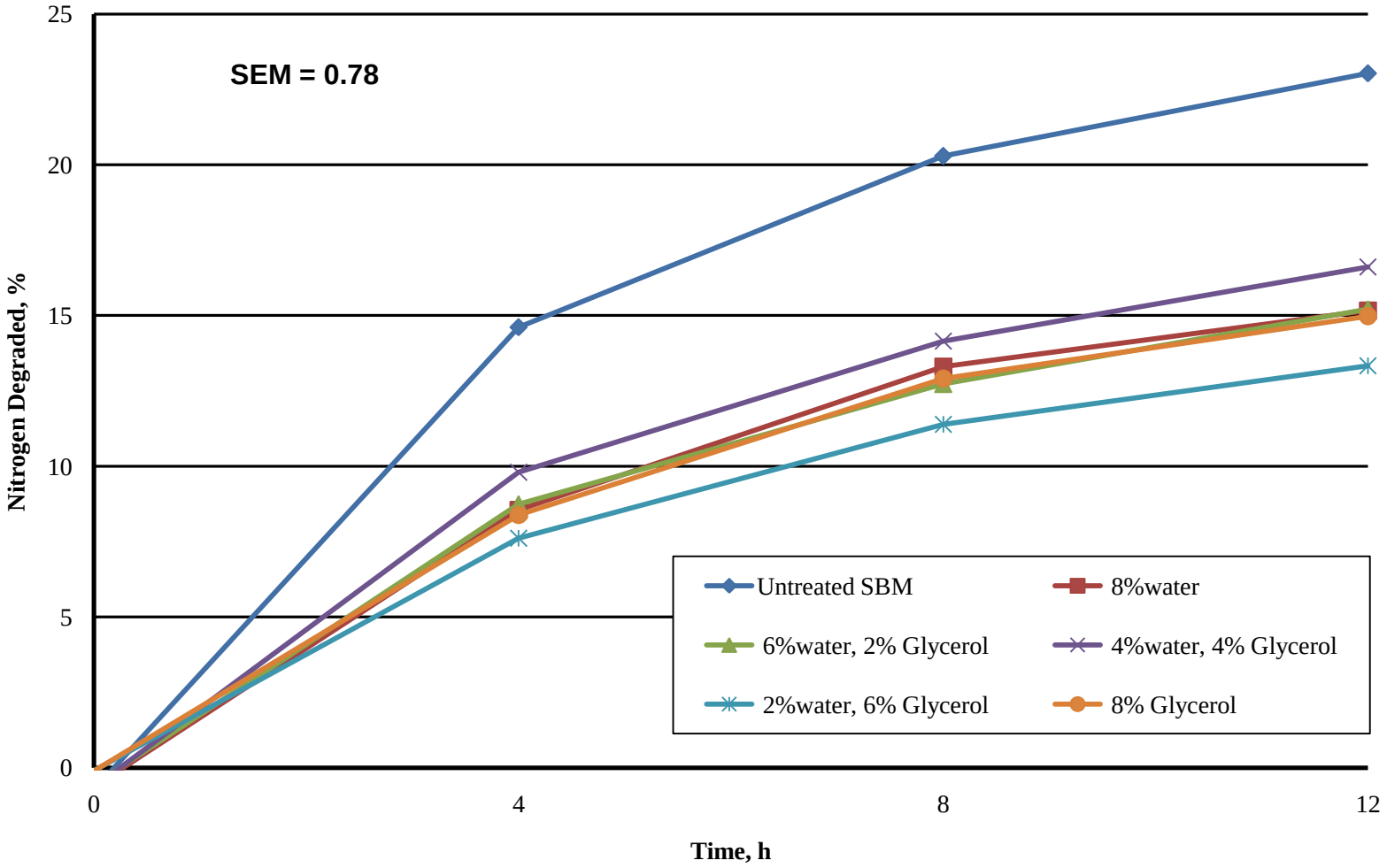
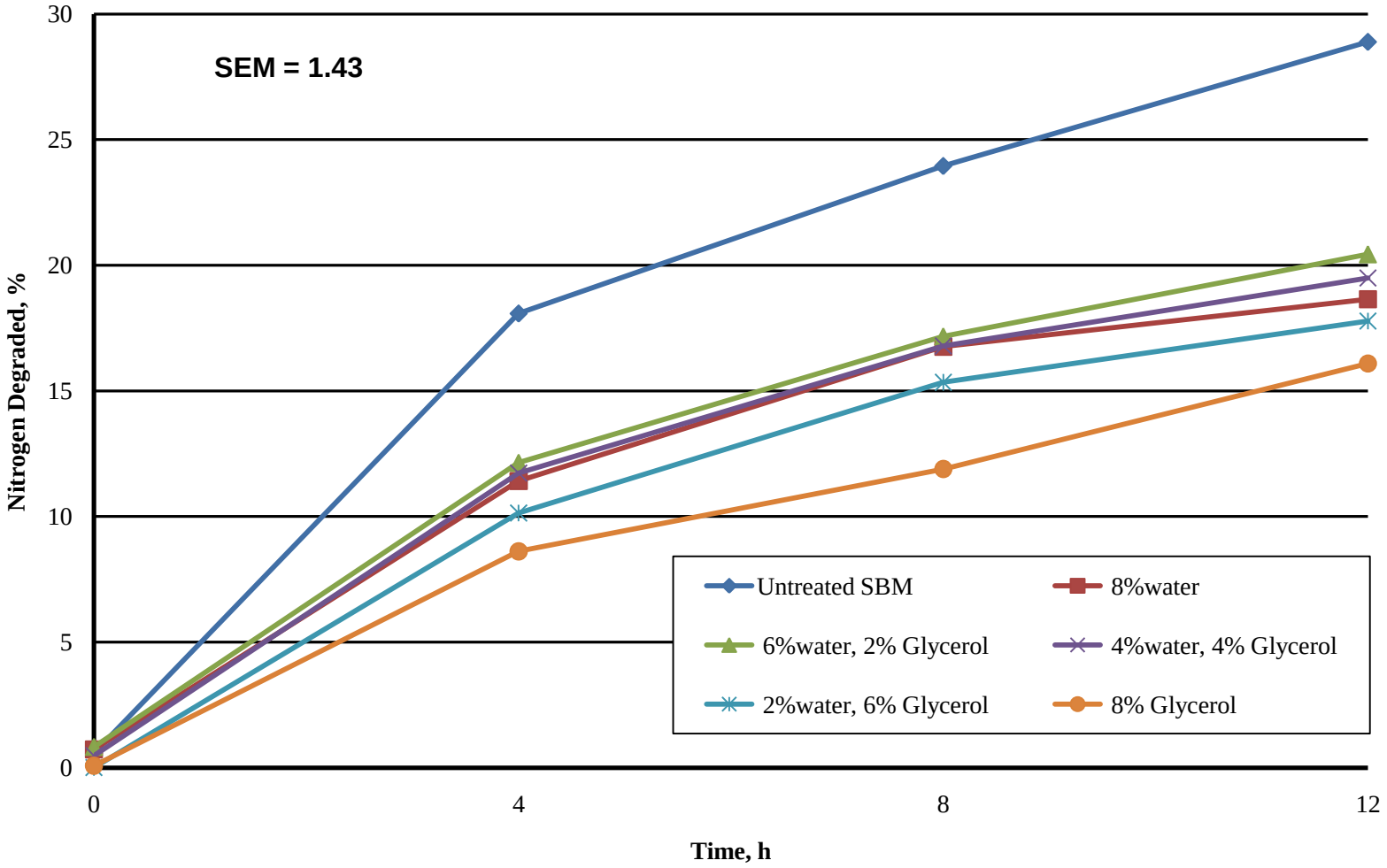
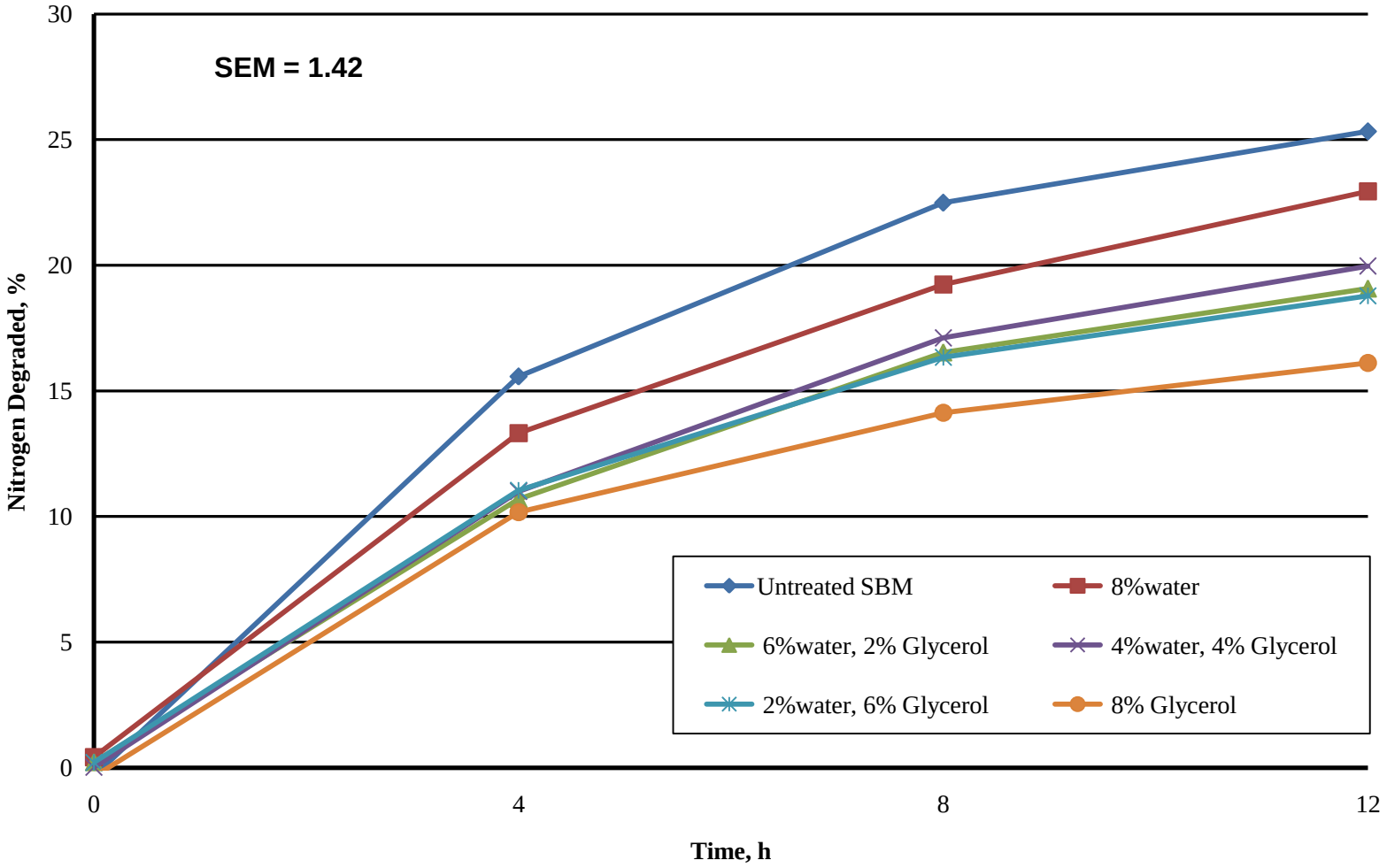


Figure 5-7. Effect of solvent on *in vitro* N degradation by ruminal microflora during a 12 h incubation period of soybean meal (SBM) products browned using invertase[a]



[a]Effect of glycerol (Linear. $P < 0.01$)

Figure 5-8. Effect of solvent on *in vitro* N degradation by ruminal microflora during a 12 h incubation period of soybean meal (SBM) products browned using yeast[a]



[a]Effect of glycerol (Linear. $P < 0.01$)