THE EFFECTS OF FEEDING DRY DISTILLER'S GRAINS WITH SOLUBLES ON RUMINAL METABOLISM, GROWTH PERFORMANCE, AND CARCASS TRAITS OF FEEDLOT CATTLE

by

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Abstract

Three trials were conducted using dried distiller's grains with solubles (DDGS) to evaluate effects on feedlot performance, carcass characteristics, ruminal fermentation, and diet digestibility in cattle fed steam-flaked corn-based diets. In trial 1, crossbred yearling heifers were used in a finishing trial to evaluate interactions between corn-DDGS feeding levels and roughage source (alfalfa hay vs corn silage) in terms of impact on feedlot performance and carcass characteristics. Experimental diets were based on steam-flaked corn and contained 0% DDGS with 6% alfalfa hay (AH), 0% DDGS with 10% corn silage (CS), 25% DDGS with 6% AH, or 25% DDGS with 10% CS (DM basis). Results indicated no interaction between levels of DDGS and roughage source. Heifers fed DDGS as a partial replacement for steam-flaked corn had similar growth performance and carcass merit compared to heifers fed diets without DDGS. Corn silage and alfalfa hay were comparable roughages when a portion of steam-flaked corn was replaced with DDGS. The second trial was a companion metabolism study in which ruminal fermentation characteristics and diet digestibility were examined in 12 cannulated Holstein steers fed steam-flaked corn finishing diets with or without DDGS, using alfalfa hay or corn silage as roughage sources. Diets were similar to those fed in the performance study and consisted of steam-flaked corn with 0 or 25% DDGS (DM basis) and 6% AH or 10% CS (DM basis). Feeding DDGS decreased ruminal pH and ruminal ammonia concentrations, and digestion of DM and OM were less compared to diets without DDGS. The decrease in digestibility was largely attributable to poorer digestion of CP and, to a lesser extent, a reduction in starch digestion. The third study was designed to investigate effects of pH (5.0, 5.5, and 6.0) on *in vitro* fermentative activity by ruminal microorganisms from cattle adapted to a finishing diet containing 25% DDGS (DM basis). Higher pH led to greater dry matter disappearance *in vitro* ($P < 0.01$). DDGS can be an effective substitute for steam-flaked corn. Efforts to address low ruminal pH and low ruminal ammonia may prove beneficial for improving value of DDGS as cattle feed.

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Dedication

I dedicate this dissertation to my heavenly Father. I thank God for answering my prayers in allowing me to start and finish my master's program. His presence sustained me all the way. He has been my all in all. To Him belong all the glory, honor, and power.

"Commit thy way unto the Lord; trust also in Him, and He shall bring it to pass." Ps 37:5

CHAPTER 1 - Literature Review

Introduction

The production of ethanol from starch or sugar-based feedstocks is among man's earliest innovations in value-added processing. Traditionally, ethanol was produced mainly for the beverage liquor industry, but it also has been used in the USA as an alternative fuel since the early 1900's (Dipardo, 2000). An early publication by Henry (1900), as cited by Klopfenstein et al. (2007), reported that by the late 19th century the main ethanol byproduct, dried distiller's grains plus solubles (DDGS), was being used as a feedstuff. The automaker Henry Ford adjusted a carburetor of the early Ford Model T to allow the vehicle to run on either gasoline or ethanol produced by American farmers. His idea was to build a vehicle that was affordable to the working family and powered by a fuel that would boost the rural farm economy (Kovarik, 1998). However, the production of ethanol from corn to fuel vehicles has repeatedly encountered commercial viability obstacles because of the abundant and inexpensive supply of fuel from petroleum and natural gas (Bothast and Schlicher, 2005).

Ethanol Production in the USA

Although early efforts to sustain the production of fuel ethanol in the USA failed, ethanol production has incredibly increased in recent years due to a number of factors. The volatility of crude oil prices due to an increasing demand, uncertainty of oil supply due to geopolitical tensions in the Middle East, and the phase-out of lead as an octane booster for gasoline are among the major reasons for the promotion of fuel ethanol production in the United States (Nguessan, 2007). According to the U.S. Department of Energy (DOE), U.S. crude oil imports reached a record of more than 10 million barrels per day in 2005, which represents 25% of the

world's total demand for oil. A half billion dollars per day were spent in 2006 on the importation of oil (DOE, 2007).

Emissions of carbon dioxide from combustion of fossil fuels are one of the main sources of air pollution and degradation of the environment. The U.S.A, with less than 5% of the world's population, was accountable for about 22% of the world's emission of carbon dioxide in 2004 (N'Guessan, 2007). In an effort to remedy to this situation, the Energy Policy Act of 2005 imposed a minimum requirement of renewable fuels to be mixed with gasoline sold for the next six years. This law mandated a minimum of 7.5 billion gallons of renewable fuel by 2012 (DOE, 2007). To achieve this goal, the federal government is sponsoring the production of fuel ethanol because it boosts the octane rating of gasoline and burns cleaner in combustion engines. Furthermore, because ethanol has a higher oxygen content than methyl tertiary butyl ether (MTBE), only half the volume is required to produce the same oxygen level in gasoline; and it is biodegradable (Dipardo, 2000). Today, ethanol production requires 50% less energy than what was required in the late 1970's (Bothast and Schlicher, 2005).

Annual production of ethanol has increased from 1,630 million gallons in 2000 to 4,855 million gallons in 2006, representing 198% growth over the period considered, and it is expanding even in states outside of the Corn Belt (Renewable Fuels Association, RFA, 2008). Fuel ethanol production currently is 5,912 million gallons per year (mgy), and expansion of existing ethanol plants or construction of new facilities will add another 6,605 mgy. This would be a total U.S. production of 12,517 mgy of ethanol (RFA, 2008).

Current Ethanol Production Processes

Ethanol can be produced from diverse sources, including grain (corn, grain sorghum, barley wheat, etc.), sugarcane, brewery by-products, or from lignocellulosic-biomass such as

wheat straw, corn stover, switch grass, etc. (Nguessan, 2007). Corn grain is the main feedstock used in the production of ethanol in the U.S. because of its high fermentable starch content compared to other grains. Starch is the major carbohydrate storage product in corn kernels comprising 70-72% of the kernel weight on a DM basis (Bothast and Schlicher, 2005). There are two ethanol production processes: wet milling and dry milling. The main difference between the two is the initial treatment of the grain which determines the initial cost of capital investments. Wet milling is more capital intensive than dry milling because the grain must first be separated into its components, including starch, fiber, gluten, and germ. Hence, wet milling requires more equipment and more energy for the production of ethanol compared to dry milling (Bothast and Schlicher, 2005). As of January 2007, dry mill facilities accounted for 82% of ethanol production and wet mills 18% (RFA, 2008).

Wet milling typically is used to produce corn oil and corn sweeteners, but starch can be fermented to produce ethanol (Schingoethe, 2006). The germ is removed from the kernel and corn oil is extracted from the germ. The remaining germ meal is added to the bran to form corn gluten feed. Gluten also is separated to become corn gluten meal, a high-protein animal feed. In the wet milling process, a starch solution is separated from the solids and fermentable sugars are produced from the starch. These sugars are fermented to ethanol (Bothast and Schlicher, 2005).

Unlike wet milling, in traditional dry milling the entire corn kernel or other starchy grain is first ground into flour referred to as "meal". The meal is mixed with water to form a "mash". Enzymes are added to the mash which is cooked at a high temperature to convert the starch to a simple sugar (dextrose) and to reduce bacteria levels prior to fermentation. Ammonia is added to control pH and as a nutrient for yeast. The mash is cooled and yeast is added to promote fermentation of sugar into ethanol and carbon dioxide. The fermentation process lasts 40 to 50

hours, yielding a mixture of ethanol and solids as end products (RFA, 2008). This mixture is then distilled and dehydrated to create 95% and 100% ethanol, respectively. Liquid removed from the mash is called thin stillage or "sweet water." Thin stillage can either be sold directly as livestock feed or dehydrated to produce condensed distiller's solubles (CDS), or “syrup.” The remaining solid fraction, called wet distiller's grains (WDG), may be sold directly as livestock feed or dehydrated to produce dried distiller's grains (DDG). Condensed distiller's solubles are either sold directly as cattle feed or blended with the distiller's grains to produce distiller's grains plus solubles (DGS). Distiller's grains plus solubles are sold in wet (30% DM), modified (50% DM), or dry forms (90% DM; Kent and Wright, 2002). Typically, 100 kg of corn yields about 40.2 L of ethanol, 32.3 kg of DDGS, and 32.3 kg of carbon dioxide (Schingoethe, 2006). Diagrams of dry milling and wet milling processes are shown in Figure 1.1.

Nutrient Content of Dry Milling Byproducts

Distiller’s Grains

Because starch, which constitutes 2/3 of the kernel, is extracted during the fermentation process, the non-starch nutrients in distiller’s grains are about three times more concentrated than the nutrients in the original grain (Linn and Chase, 1996). Distiller’s grains are low in starch, but high in fat, protein, fiber, and phosphorus. Wet and dry distiller’s grains have similar chemical composition but vary in dry matter content (wet distiller’s, 35-45%; dried distillers, 90-95%, Klopfenstein et al., 2007). They contain 10-15% ether extract, 40-45% NDF, 30-35% CP, and 5% ash (NRC, 1996). Distiller’s grains without solubles have lower phosphorus content (~0.4%) compared to distiller’s grains with solubles because solubles have high phosphorus content (~1.35%). Protein and fat content of distiller’s grains with solubles are usually slightly higher compared to distiller’s grains without solubles (Schingoethe, 2006).

Because most of the readily degradable proteins in corn have been degraded during the fermentation process, the protein remaining in the distiller's grains is proportionately higher in ruminal undegradable protein (RUP) than in the original corn. Aines et al. (1987) summarized reports on rumen protein escape values of DDGS and found average escape values to be 2.6 times soybean meal and values for dry distiller's grains without solubles (DDG) were 2.3 times that of soybean meal. Dong et al. (1987) evaluated the amino acid profiles of several wheat DDGS. Their results suggested that amino acid profiles in the DDGS are similar to the whole grain before fermentation.

In addition to protein, NDF is more concentrated in DDGS compared to the original corn grain. Sayer et al. (2005) reported NDF content of the corn bran to be 69%, and its extent of *in situ* digestion varied from 79 to 84% in cannulated cattle fed finishing diets. Rates of NDF digestion in these finishing diets were 1.7 to 2.1%/h. In a study by May (2007) evaluating DDGS in diets comprised of dry-rolled corn or steam-flaked corn, feeding DDGS did not affect NDF digestibility in type of diet. Because of their high content of fiber and fat compared to the original corn grain, wet DGS was reported to contain 29 to 40% more NE_{gain} than dry rolled-corn, whereas dried DGS contained only 21% more NE_{gain} than dry rolled-corn (Ham et al., 1994).

Distiller's grains are low in Ca but high in P and S (Kent and Wright, 2002). Sulfuric acid is used to control pH and to clean fermentation equipment, resulting in S levels of 0.6 to 1.0% or greater in DGS (Klopfenstein et al., 2007). While S is required by ruminal microorganisms, high levels (above 0.4% DM) may cause polioencephalomalacia, reduce DMI and ADG, and reduce liver Cu stores in cattle (Loneragan et al., 2001). Sulfur interferes with Cu absorption and metabolism (Kent and Wright, 2002).

Feedlot diets generally contain excess phosphorus due to the high levels of P in corn grain. Thus, one must consider disposal of the additional P excreted in manure when DGS are fed. Although there is no evidence that high levels of P are detrimental to feedlot cattle, Ca:P ratios must be equal to or greater than 1.2:1, but not greater than 7:1(NRC, 1996) to facilitate adequate performance and to avoid urinary calculi (Kent and Wright, 2002).

Information available about the nutrient content of DDGS produced from the fermentation of other grains such as wheat, sorghum, or barley is limited. However, data available indicate that the composition usually reflects the nutrient content of the grain before fermentation (Schingoethe, 2006).

Condensed Distiller's Solubles

Condensed distiller's solubles (CDS) contain up to 15% fat depending on the source (Kent and Wright, 2002). Feeding CDS provides additional protein and energy and add moisture to condition diets. However, much of the protein in CDS is yeast cells which have been heated during distillation and concentration (Klopfenstein et al., 2007). Yeast concentrations often reach 150 million cells per cubic centimeter in mashes after 26 hours of fermentation (Hatch, 1995). Heat denaturation renders CDS resistant to lyses and microbial degradation (Bruning and Yokoyama, 1988). Research by Herold (1999) suggests that only 20% of CDS from the wet milling are degradable in the rumen.

Gilbery et al. (2006) fed corn condensed distiller's solubles (CCDS) at levels of 0, 5, 10 or 15% diet DM as a protein source to cattle fed poor quality hay. Their results showed that OM intake, total duodenal OM flow, microbial flow, non microbial flow, and fecal OM flow increased linearly as CCDS was added to the diet. Similarly, a linear increase in duodenal CP

flow: microbial, total CP and fecal CP output was observed as CCDS increased in dietary percentages. Total tract digestibility was higher for cattle fed high levels of CCDS in the diet. Research by Rust et al. (1990) investigated the effect of CCDS as an energy source in finishing steers. Steers were fed either grain soaked in CCDS, CCDS added to water or *ad libitum* CCDS, but the latter group was not allowed free access to water. No differences among treatments with regard to DMI and ADG were observed. Steers consuming CCDS *ad libitum* had higher feed efficiency compared to the control groups. Likewise, metabolizable energy was greater for cattle that had *ad libitum* supplement of CCDS compared to the control treatment not fed CCDS. Conversely, ruminal butyrate concentrations increased in cattle fed corn that was soaked in CCDS compared to other treatment groups. Fron et al. (1996) investigated the effects of feeding CCDS in dry-rolled corn diets on ruminal microbiology and metabolism. Ruminal lactate was higher in cattle fed CCDS compared to those fed no CCDS. Including CCDS into diets increased cultural lactilytic bacteria and amylolytic bacteria but decreased total protozoal counts. These researchers suggested that adding CCDS early in the feeding phase can promote growth of lactilytic bacteria and thus decrease ruminal lactate.

Thin Stillage

Thin stillage contains only 5-10% DM and can be used to replace water in cattle feeding operations. Replacing water with thin stillage reduced DMI without negatively affecting performance (Kent and Wright, 2002).

Variability of Nutrients Composition of Ethanol Byproducts

Although basic steps for ethanol production are the same across ethanol plants, the variability of nutrient content of ethanol byproducts continue to raise concerns in formulating beef diets that contain DGS, either wet or dry. Chase (1991) demonstrated ranges in nutrient

content (% DM) of DDGS as follows: 22 to 33% CP, 29 to 64% NDF, and 2 to 20% ether extract. Spiehs et al. (2002) evaluated nutrients content of DDGS in 10 plants in Minnesota and South Dakota. They collected samples every two months between 1997 and 1999. Their results showed the following averages and coefficients of variation: 88.9% DM with CV of 1.7%; 30.2% CP with CV of 6.4%; 10.9% ether extract with CV of 7.8%; 8.8% crude fiber with CV of 8.7%; 5.8% ash with CV of 14.7%, 16.2% ADF with CV of 28.4%; 42.1% NDF with CV of 14.3%; 0.06 % Ca with CV of 57.2%; and 0.89 % P with CV of 11.7%. Likewise, Buckner et al. (2008) analyzed WDGS from 6 ethanol plants in Nebraska between summer 2006 and winter 2007 to determine nutrient variability. The average content and coefficients of variation within plants were as follow (%DM): 11.8% ether extract with CVs of 1.9 to 8.8 %; 31% CP with CV of 1.3 to 3.9 %; 0.79% sulfur with CVs of 3.5 to 36.3%; and 0.82% phosphorus with CVs ranging from 1.3 to 6.0%. The above coefficients of variation clearly demonstrate the large variation in nutrient content of the distiller's grains from plant to plant as well as within plants. The major factors of variability are the type of grain, milling processes, grain quality, fermentation processes, drying temperatures and proportion of solubles blended back into the unfermented fraction at the time of drying (Linn and Chase, 1996).

The ruminal undegradable protein content is of particular interest because prolonged exposure to high temperatures and reducing sugars may result in a chemical "browning reaction" that renders part of the carbohydrate and protein unavailable to the animal. The Maillard reaction may be one of the major sources of variation in protein availability of dried and modified distiller's grains since they are subjected to a drying process. According to NRC (1996), RUP of corn DDGS is 52% of the CP. Other researchers have shown RUP of corn-based DDGS to vary from 45% (Powers et al., 1995) to 55% (Grings et al., 1992). Stern et al. (1995) analyzed

samples of 5 distiller's grains and found a RUP of 56 ± 8% with an intestinal digestibility of the RUP fraction at 81 ± 5%. According to Chase (1991), soluble intake protein (SIP) of distiller's grains is about 15% the CP, but Powers et al., (1995) have observed it to be 28.5% of the CP. To accurately estimate the nutritional value of the ethanol byproducts, each load must be sampled and tested. Acid detergent insoluble nitrogen (ADIN) is commonly used in many commercial laboratories as an estimate of N digestibility for protein sources (Ham et al., 1994; Kent and Wright, 2002). However, research by Nakamura et al. (1994) suggested that ADIN was a poor indicator of protein damage in nonforage protein supplements.

Effects of Distiller's Byproducts on Feedlot Performance

Distiller's grains can be fed at 6 to 15% DM in feedlot finishing diets primarily as a source of supplemental protein, or at higher levels (greater than 15% DM) as a source of energy replacing corn grain (Klopfenstein et al., 2007). Numerous studies have been conducted comparing DGS from different grain types, wet vs dry distiller's gains, and evaluating interactions with grain processing methods, interactions with roughage source and roughage level.

Distiller's Grains from Different Grains

Although corn is the primary grain used for ethanol production in the USA, grain sorghum is an attractive feedstock for ethanol plants because it is less expensive than corn. Both grains have similar amounts of starch and therefore yield similar amounts of ethanol (Klopfenstein et al., 2007). Lodge et al. (1997) compared sorghum distiller's grains with or without solubles to corn distiller's grains with or without solubles. All feedstuffs were fed at 40% DM in dry rolled-corn (DRC)-based finishing diets. There were no differences among treatments with regard to DMI and ADG. Cattle fed no distiller's grains and cattle consuming

sorghum WDG or sorghum WDG plus solubles had similar feed efficiencies. Al-Suwaiegh et al. (2002) compared sorghum and corn DGS made at the same ethanol plant. Although the feed efficiencies were not different, values for corn DGS were numerically higher than those for sorghum DGS. Depenbusch et al. (2007a) found similar results when they compared sorghum vs corn-based DGS in steam-flaked corn (SFC)-based finishing diets. Vasconcelos and Galyean (2007) reported statistically similar responses in G:F for sorghum and corn DGS (0.169 and 0.176, respectively).

Wheat is used as a feedstock in ethanol plants of Western Canada due to its availability compared to corn (Klopfenstein et al., 2007). Wheat DGS has more NDF and less fat than corn DGS, whereas the protein is more degradable (Mustafa et al., 2000).

Wet vs Dry Distiller's Grains

Distiller's grains plus solubles, wet or dried, are routinely fed as a source of energy and protein in feedlot diets. In a study by Ham et al. (1994), feed efficiency was improved 9% when DDGS replaced 40% of the dry-rolled corn in finishing diets. However, this improvement was only 50% of that observed when wet distiller's byproduct replaced the same amount of DRC. The author suggested that drying process appears to reduce the energy value of the distiller's byproduct. However, since DM was determined by oven drying, it is conceivable that some volatile organic matter was lost and contributed to this difference.

Larson et al. (1993) fed 5.2, 12.6 or 40% WDGS (DM) as a partial replacement for DRC in finishing diets. With the first two levels (5.2 and 12.6), feed efficiency was 7% improved over that observed in the control group fed no WDGS. But, when the inclusion level was increased to 40% DM, the improvement in feed efficiency was 20% above the DRC diet. Likewise, Lodge et

al. (1997) measured a 15 to 25% improvement in feed efficiency when 30 to 40% of the DRC was replaced by WDGS.

Buckner, et al., (2007) conducted a feedlot study comparing 10, 20, 30, and 40% levels of DDGS to a DRC control. A trend for a quadratic response was observed for G:F. The quadratic response in G:F was similar to that found by Vander Pol et al. (2006b) when WDGS replaced a portion of DRC. However, in this study, the G:F response was less and optimal inclusion level was 20% of diet DM.

Five studies investigating DDGS and eleven trials evaluating WDGS were summarized where DGS was compared to corn as an energy source for finishing cattle. The DDGS analysis revealed that ADG increased quadratically while G:F followed a cubic response as level of DDGS in the diet increased from 0 to 40%. Average daily gain was higher between 20 to 30% DDGS while the highest G:F was attained between 10 to 20% DDGS. The WDGS analysis showed a quadratic response with respect to ADG and DMI. The highest values were obtained with an inclusion of 30% DM. The G:F tended to increase quadratically and the highest values were attained at 30 to 50% WDGS of the diet (Klopfenstein et al., 2007). Feeding either DDGS or WDGS did not affect palatability of the meat (Roeber et al., 2005).

Because wet and dried DGS have relatively similar nutritive value, considerations regarding handling, transport and cost play an important role in deciding which DGS to feed. Since DDGS only contain 10-12% moisture, they can be shipped greater distances more economically and conveniently than wet DGS. They can be easily mixed with other ingredients and are easy to store. However, because of their small particle size, storing DDGS out of the wind may be critical. Although DDGS have high levels of fat, rancidity during summer months is usually not a concern (Schingoethe, 2006; Kent and Wright, 2002). Feeding wet DGS avoids

the cost of drying the product, but WDGS contain about 70% moisture, which limits their transport to shorter distances and makes storage more challenging. During winter, WDGS may freeze into clumps leading to inconsistency of the ration due to the poor blending capacity of the frozen WDGS. During warmer months, WDGS tend to mold, and have a short shelf life of about 7 days (Kent and Wright, 2002). Research by Spangler et al. (2005) suggests that the addition of preservatives such as propionic acid or other organic acids may extend the shelf life of WDGS. Wet distiller's grains have been stored successfully for more than 6 months in silo bags either alone or in combination with other feeds such as soy hulls (Kalscheur et al., 2002), corn silage (Kalscheur et al., 2003), beet pulp (Kalscheur et al., 2004a) and other feedstuffs to increase bulk (Kalscheur et al., 2004b).

Distiller's Grains with Grain Processing Methods

Although DGS have similar or more energy density than corn, performance of cattle fed distiller's byproducts seems to depend on the type of the grain fed. Vander Pol et al. (2006a) fed DRC, SFC, and high moisture corn (HMC) with 30% WDGS to finishing cattle. Grain processing method did not affect G:F but cattle fed SFC had a lower ADG compared to their counterparts fed DRC or HMC. However, Corrigan et al. (2007) evaluated the interaction between inclusion levels of WDGS and grain processing method. The WDGS was fed at 0, 15, 27.5, or 40% of DM and grain types were DRC, HMC, or SFC. There were interactions for ADG and G:F between levels of WDGS and grain processing type. With DRC- based diets, final BW, ADG, and feed efficiency increased linearly as levels of WDGS increased. Cattle fed SFC- based diets responded quadratically with respect to final BW and ADG, with 15% WDGS being the optimal. When WDGS was added to HMC, there was a quadratic response for ADG and a linear improvement in G:F. Similarly, research by May et al. (2007a) demonstrated poor feedlot

responses to WDGS in SFC-based diets compared to DRC-based diets. Daubert et al. (2005) evaluated inclusion of 0, 8, 16, 24, 32, and 40% WDGS (dry basis) in SFC-based diets. In this study, regression analysis of the efficiency data showed that the optimum amount of sorghum WDGS in steam-flaked corn diets was approximately 15%. Diets containing up to 24% WDGS yielded efficiencies equal or superior to diets containing no WDGS.

A study by Depenbusch et al. (2007b) showed that de-germed distiller's grains and traditional distiller's grains have similar feeding value. Reinhardt et al. (2007) analyzed 21 individual feeding studies from 6 states to determine the carcass fat distribution of feedlot cattle fed various levels of distiller's byproducts (DG). Their results indicated that feeding low levels of DG increased marbling score whereas feeding high levels of DG depressed marbling score. Feeding moderate levels of DG resulted in high marbling scores but relative change in overall body fatness was even more dramatic than changes in marbling score.

Distiller's Grains with Roughage Source and Level

Distiller's grains are a great source of nonforage fiber because they have high NDF content but low lignin content. Thus, DGS can partially replace forages and supply energy needed for growth without excesses of ruminal organic acids due to rapid fermentation of starchy grains (Ham et al., 1994). In addition to supplying NDF and reducing starch in the diet, DGS supply protein; hence low quality forage may be fed with relatively high levels of DGS (Klopfenstein et al., 2007). One might think that when DGS is included in finishing diets at levels higher than 20% of DM not only should incidence of subacute acidosis be reduced but also roughage (forage) content of the diet should be reduced. However, because of the small particle size, DGS may lack sufficient "fiber effect" (Schingoethe, 2006). Bhatti and Firkins (1995) evaluated effective fiber values of DGS. They demonstrated that the digestion of NDF in

distiller's grains is initially slow, but once initiated the digestion rate becomes relatively fast (0.0626/hour). These authors suggested that the slow initiation could be an indication of the low water holding capacity (0.062 g/g of insoluble DM) of NDF in distiller's grains, since fiber must be hydrated before digestion by bacteria. The slow initial digestion rate in conjunction with a small particle size can result in a small retention time in the rumen. Thus, the physical effectiveness of NDF in distiller's grains to stimulate cud chewing appears to be quite limited (Linn and Chase, 1996).

Benton et al. (2007) evaluated the effect of roughage level and source in DRC-based finishing diets containing 30% WDGS. Alfalfa hay was used as a reference and was fed at 4 and 8% of the diet DM, while a diet without roughage served as a control. Corn stalks were fed at 3 and 6% of the diet DM, based on NDF equivalent of alfalfa. Corn silage was also included on an equal NDF basis at 6 and 12% of the diet DM. Dry matter intake increased 1 to 1.5 kg/d due to roughage inclusion and ADG increased 0.09 to 0.22 kg/d. Shain et al. (1999) obtained similar increases in DMI and ADG without feeding WDGS, which suggests that WDGS did not supply a "roughage effect", although it supplied NDF. However, compared to alfalfa and corn silage, corn stalks provided a similar roughage effect, yielding similar DMI, ADG, and G:F when WDGS was fed. Unlike these data, Shain et al. (1999) found that wheat straw was not as efficiently utilized as alfalfa when fed on an equal NDF basis to alfalfa in dry-rolled corn diets. These results may indicate that low quality roughages may be fed in conjunction with WDGS without adverse effects on growth performance of feedlot cattle. Research by May et al. (2007c) suggests that roughage levels can be reduced in SFC-based finishing diets containing DGS without compromising efficiency, health, or carcass quality of feedlot cattle.

Ruminal Metabolism and Digestibility of Distiller's Grains in Feedlot Diets

When fed as a source of energy, distiller's grains with solubles have improved feedlot performance when fed with dry-rolled corn compared to DRC-based diets without DDGS (Larson et al., 1993; Al-Suwaiegh et al., 2002; May et al., 2007b). The major characteristics of DGS that might cause these performance differences include ruminal pH, high content of NDF, protein, and fat. A number of metabolism studies have been conducted in effort to investigate on the aforementioned factors. Feeding ethanol by-products could conceivably be beneficial in high concentrate diets since starch is extracted during fermentation and fiber content is increased. However, when Corrigan et al. (2008) fed 0 or 40% WDGS in DRC, HMC, or SFC-based diets, their results showed that feeding WDGS does not increase rumen pH, though it did decrease variance. Similarly, in a study by May (2007), ruminal pH was lower in cattle fed 25% DM DDGS (DM basis) compared to cattle fed no DDGS as a partial replacement of SFC or DRC in finishing diets. Vander Pol et al. (2007) conducted a metabolism study to determine what element of DGS is responsible for its higher energy in comparison to corn grain in feedlot diets. They fed 40% WDGS (DM basis), a composite of corn fiber and corn protein (COMP), COMP +oil, DRC as control (CON) or CON + oil. Their results suggest that WDGS does not control acidosis by increasing rumen pH; rather, the high energy value of WDGS is due to higher fat digestibility, more propionate production, and more unsaturated fatty acids (UFA) reaching the duodenum. The digestibility of added fat as corn oil was 70%, while fat added as WDGS was 81% digested. Steers fed WDGS had 21% higher unsaturated fatty acids flowing in duodenum than their counterparts fed similar amounts of corn oil. Poor digestion of saturated fats could explain this negative influence. According to Plascencia et al. (2003), intestinal fatty acid digestion decreased with level of total fatty acid intake, regardless of degrees of saturation. Feeding DDGS in DRC or SFC-based finishing diets resulted in lower total tract digestibility of

ether extracts compared to feeding no DDGS (May, 2007). The negative effect of fat on rumen fermentation has been demonstrated (Zinn et al., 2000) and may be additive to the decreased digestion of fat.

May (2007) evaluated the digestibility of DDGS in diets comprised of SFC or DRC. Cattle consuming DDGS tended to have lower apparent total tract digestibility of DM and OM compared to cattle without DDGS in either grain processing method. Similarly, in a study by Depenbusch et al. (2007b), digestibility of DM, and OM were decreased by adding approximately 13% DDGS or de-germed corn dried distiller's grains with solubles to SFC diets. In the study by May (2007), ruminal lactate concentration increased with addition of DDGS compared to diets without DDGS. Steers fed 25% DDGS also had lower ruminal ammonia concentrations than steers fed 0% DDGS during the first 10 hours after feeding. Feeding DDGS in DRC-based diets resulted in a lower magnitude of change in digestibility compared to feeding DDGS in SFC-based diets.

Conclusion

Availability of distiller's grains as a feed for ruminants likely will increase as the fuel ethanol industry expands. Distiller's grains are rich in fiber, protein and fat. Distiller's grains can be fed as protein source when fed at < 15% DM or as an energy source when included at levels greater than 15% DM in finishing diets. The effects of distiller's grains on feedlot performance are not influenced by the type of the grain fermented or physical form (wet vs dry) of distiller's grains. However, performance is influenced by processing method of the basal grain. Although the ethanol production process is similar across production facilities, nutrient variability of distiller's grains is still a source of concern.

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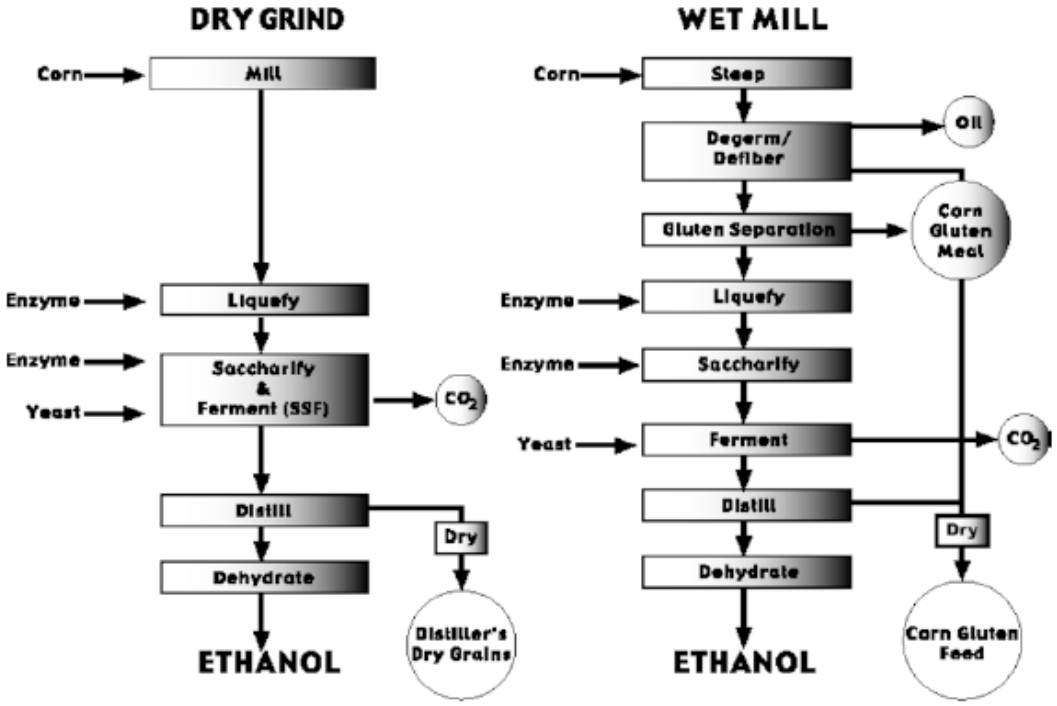
Figure 1-1 Dry and wet milling ethanol production processes



Source: Bothast and Schlicher, 2005

CHAPTER 2 - Evaluation of dried distiller's grains and roughage source in steam-flaked corn-based finishing diets

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Abstract

A finishing trial was conducted to investigate the use of dried corn distiller's grains with solubles (DDGS) in steam-flaked corn (SFC) diets using corn silage (CS) or alfalfa hay (AH) as roughage sources. Experimental diets (DM basis) consisted of SFC and 10% CS without DDGS (SFC_CS); SFC and 10% CS with 25% DDGS (DDGS_CS); SFC and 6% AH without DDGS (SFC_AH); and SFC with 25% DDGS and 6% AH (DDGS_AH). Crossbred heifers (n=358; BW = 353 ± 13 kg) were individually weighed and assigned to 24 dirt surfaced pens containing 14 to 15 animals each, with six pens per treatment. Heifers were fed for *ad libitum* intake once daily for 97 d. Feeding DDGS did not affect ADG, DMI, or feed conversion. Within roughage source, heifers fed CS had greater DMI than those fed AH ($P < 0.05$) but ADG and G:F were not affected ($P > 0.10$). There were no differences among treatments with respect to carcass weight, dressing percentage, subcutaneous fat thickness, quality grades, or yield grades 1, 3, 4, and 5 ($P > 0.20$). Overall, heifers were relatively lean. The average yield grade for all treatment groups was near to 2. Cattle fed CS tended to have higher ($P = 0.10$) marbling score than their counterparts fed AH. There was an interaction between roughage and DDGS levels with respect to incidence of liver abscess ($P = 0.02$). The highest incidence was observed in cattle fed SFC_CS while the lowest was observed in cattle fed SFC_AH. Partial replacement of SFC with DDGS did not affect cattle performance, carcass quality or yield grades. Additionally, CS and AH can be fed interchangeably in finishing diets, when a portion of SFC is replaced with DDGS.

Introduction

Ethanol production in the U.S. is expanding rapidly in response to provisions of the Energy Policy Act of 2005, hence increasing the availability of distiller's grains (Renewable Fuels Association, 2005). Distiller's grains with solubles are the main by product of the dry milling industry and are good source of protein (> 30% CP) and energy (10% fat; NRC, 1996). These co-products are commonly added to finishing diets in the wet (WDGS) or dry (DDGS) forms to replace portions of the cereal grains and protein sources in cattle diets.

Roughages are included in feedlot diets to help prevent digestive disorders and to maximize NE intake by cattle. Finishing diets typically contain between 4.5 and 13.5% (DM basis) roughage, with alfalfa and corn silage being the most common sources (Galyean and Defoor, 2003). Previous research indicated that roughage effects on daily gains and feed efficiencies of feedlot cattle varied based on grain type (Gill et al., 1981; Theurer et al., 1999; Mader et al., 1991). Partial replacement of cereal grains with distiller's grains has been extensively studied, revealing diverse results depending on the type and amount of DGS as well as roughage source used (Al-Suwaiegh et al., 2002; May et al., 2007a; May et al., 2007b). Specifically, in a study by May et al. (2007a) feeding dried distiller's grains with solubles (DDGS) in steam-flaked corn finishing diets decreased cattle performance when added to diets containing alfalfa hay but this negative effect was not observed in a subsequent study when DDGS was fed using corn silage as the roughage source (May et al., 2007b).

The objective of this study was to test interaction between DDGS and roughage source by directly comparing the effect of corn silage or alfalfa hay as the roughage sources in steam-flaked corn diets containing dried distiller's grains with solubles.

Materials and Methods

Procedures were approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2315.

Three hundred and sixty crossbred yearling heifers (353 ± 13 kg initial BW) were fed flaked-corn finishing diets containing 0 or 25% DDGS (DM basis) with AH or CS to evaluate effects on growth performance and carcass characteristics of finishing beef heifers. The study consisted of a randomized complete block design with a 2 x 2 factorial arrangement of treatments. Experimental diets consisted of steam-flaked corn (SFC) and 10% CS without DDGS (SFC_CS); SFC and 10% CS with 25% DDGS (DDGS_CS); SFC and 6% AH without DDGS (SFC_AH); and SFC with 25% DDGS and 6% AH (DDGS_AH). The four dietary treatments used were based on corn steam-flaked to a bulk density of 360g/L. Diets were formulated to be isonitrogenous at 14% crude protein (CP). Composition of experimental diets is further described in Table 2-1. Diets were mixed once daily immediately prior to feeding, and the weights of fresh feed provided as well as feed removed were recorded. Feedstuff were sampled weekly and analyzed for DM, CP, according to Understander et al. (1993) and AOAC (1995) official method 990.03 and starch availability using a refractive index according to Sindt et al. (2000).

Upon arrival at the KSU feedlot, heifers were fed ground alfalfa hay and water for *ad libitum* intake. One day after arrival, they were identified by using one ear tag that displayed a unique number for each study animal. Before the initiation of the study, heifers were individually weighed and they received an estradiol/trenbolone acetate implant (Revalor 200, Intervet, Inc.), a topical parasiticide (Phoenectin pour-on, IVX Animal Health, St. Joseph, MO), a 4-way viral vaccine (Bovishield–IV, Pfizer Inc.), and a 7-way clostridial vaccine (Fortress – 7, Pfizer, Inc.).

Heifers were then blocked by weight and randomly assigned within block to treatments and pens. Twenty four pens were used in this study, with six pens per treatment, and 15 animals

per pen. Due to reasons unrelated to treatments, two heifers did not finish the study. As a result, two pens contained only fourteen heifers at the end of the study. Four step-up diets were fed *ad libitum* for five days each before feeding the respective finishing diets (Table 2.1). Step-up diets were formulated to offer a gradual replacement of a portion of alfalfa hay with steam-flaked corn at each step. Likewise, alfalfa hay (AH) was gradually replaced with corn silage (CS) in diets that were formulated to contain CS as a roughage source (SFC_CS and DDGS_CS).

Heifers were housed in dirt-surfaced outdoor pens located at KSU Beef Cattle Research Center. This facility consists of pens that are 9.9 m wide x 24 m deep. Pens provide for 45 to 50 cm of bunk space per animal. Pens were equipped with fence-line water fountains that are shared between adjacent pens. Rations were delivered to each pen once each day in quantities that result only in traces of residual feed in the bunk of the following day. The water was municipal and was available for *ad libitum* intake. Cattle were observed once daily from trial initiation until slaughter and general health observations were recorded.

Heifers were harvested on day 97 and final weights were determined immediately before cattle were brought to a commercial slaughter facility at Emporia, KS. Hot carcass weight and incidence and severity of liver abscesses were recorded the day heifers were slaughtered. The incidence and severity of liver abscesses were scored according to the Elanco scoring system – A^- = one or two small abscesses or abscess scars, A = two to four small well-organized abscesses, and A^+ = one or more large or active abscesses with or without adhesions. LM area; subcutaneous fat thickness over 12^{th} rib; KPH fat; marbling score; USDA yield grades; and USDA quality grades were determined following a 24-h chill. The final body weight was determined as hot carcass weight divided by a common dressing percentage of 63.5%. Average daily gains were computed by subtracting initial live weight from carcass adjusted final body

weight divided by days on feed. Gain efficiencies were computed by dividing average daily gain by average daily feed intake on a dry matter basis.

Growth performance, carcass characteristics, USDA yield grades and quality grades were analyzed statistically using the Mixed Procedure of SAS version 9.1 (SAS Inst. INC., Cary, NC). Pen was the experimental unit, and weight block was used as the random effect. The model statement included DDGS level, roughage source, and the interaction between these two main effects. Treatments averages were determined by using LSMEANS. A $P \leq 0.05$ was considered significant.

Results and Discussion

Growth Performance

Growth performance data are summarized in Table 2.2. There was no roughage x DDGS level interaction ($P > 0.20$) with respect to DMI, ADG, and G:F. The replacement of SFC with 25% of DDGS did not affect ($P > 0.10$) DMI, ADG, or G:F. Consequently, dietary NEm and NEg calculated based on the animal performance were similar ($P > 0.20$) across treatment groups.

Similar to our results, in research by Lodge et al. (1997) and Al-Suwaiegh et al. (2002), DMI was not affected when cattle were fed 30 and 40% (DM basis) of sorghum and corn distiller's grains, respectively, in finishing diets comprised of dry-rolled corn (DRC). Likewise, when May et al. (2007b) fed 25% DDGS (DM basis) in SFC diets, DMI was not affected. Conversely, Larson et al. (1993) obtained a linear decrease in DMI when they increased the inclusion of wet distiller's grains from 0 to 40 % of the diet DM. Unlike our results, Al-Suwaiegh et al. (2002) demonstrated a 10% improvement in ADG while Larson et al. (1993) observed a tendency for 6% improvement in ADG. In accordance to our results, May et al.

(2007b) found no effect on ADG. Both Larson et al. (1993) and Al-Suwaiegh et al. (2002) demonstrated an improvement in feed efficiency when they fed up to 40% of distiller's whereas Lodge et al. (1997) and May et al. (2007b) observed no differences when 40% and 25% were fed, respectively. Our finishing diets were based on steam-flaked corn while dry-rolled corn (DRC) was the main ingredient for Larson et al. (1993), Lodge et al. (1997), and Al-Suwaiegh et al. (2002). According to NRC (1996) NEg is 4% higher for steam flaked corn compared to dry rolled corn. It is conceivable that partial replacement of SFC with DDGS did not increase dietary energy density in our study in contrast to increases in energy density when distiller's grains replaced DRC in studies by Larson et al. (1993), Lodge et al. (1997), and Al-Suwaiegh et al. (2002). Moreover, it is known that cattle fed SFC finishing diets have lower rumen pH compared to those fed DRC finishing diets (Barajas and Zinn, 1998; Corona et al., 2006; May, 2007). It is also well documented that the optimal pH range for fibrolytic bacteria is usually between 6.0 and 6.5 (Huang et al., 1988; McGavin and Forsberg, 1988; McGavin et al., 1989) and hence their activity declines at a lower pH (Russell and Wilson, 1996). Because finishing beef cattle fed a steam-flaked corn finishing diet have a ruminal pH that is usually below 6 (Zinn, 1990; Adam, and Tamayo, 1995; Sindt et al., 2006) it is probable that including DDGS reduced gain energy due to relatively lower digestibility of the diet containing distiller's grains. Results from our companion metabolism study, in which cattle were fed diets similar to those fed in this study, indicated that apparent total tract digestibility of OM was lower in cattle fed DDGS compared to their counterparts fed diets without DDGS. This was likely due to low ruminal pH (< 5.5) observed in all treatment groups for more than 12 h after feeding. Additionally, in the metabolism study, cattle fed 25% DDGS had a lower apparent total tract digestibility of CP compared to their cohorts fed diets without DDGS. Because DDGS replaced not only a portion

of corn, but also urea in the diets, it is conceivable that N availability in rumen became a limiting factor to ruminal microbial growth and subsequent digestion of the substrate (Bach et al., 2005). In spite of low digestion of diets containing 25% DDGS, feed conversions of heifers fed diets with DDGS were similar to those of heifers fed no DDGS. This may be attributable to slightly higher dietary fat concentration for cattle fed diets containing DDGS compared to heifers fed no DDGS. According to Vander Pol et al. (2007), the high energy value of distiller's grains with solubles is due to higher fat content and digestibility, more propionate production, and more unsaturated fatty acids (UFA) reaching the duodenum.

Within roughage source, heifers fed corn silage had greater (P = 0.05) DMI than those fed alfalfa hay. Nonetheless, feeding either corn silage or alfalfa hay as roughage source had no effect on ADG, G:F, NEm, and NEg. Gill et al. (1981) evaluated five roughage levels (8, 12, 16, 20, and 24% of DM) in diets based on high-moisture corn, steam-flaked corn, or a 50:50 mixture (DM basis) of high-moisture corn (HMC) and steam-flaked corn (SFC). Roughage was a mixture of alfalfa (1/3 on a DM basis) and corn silage (2/3 on a DM basis). Across grain type, increasing roughage level increased DMI, but effects on daily gains and feed efficiencies varied based on grain type, with 8, 12, and 16% roughage being optimal for SFC, the 50:50 mixture of HMC and SFC, and HMC, respectively.

Previous research from Stock et al. (1990) and Kreikemeier et al. (1990) indicate that feeding a 50:50 mixture of corn silage and alfalfa in DRC finishing diets resulted in a linear increase for DMI as roughage level increased from 0, 3, 6, 9 % of DM and 0, 5, 10, or 15% of DM respectively. In both studies ADG responded quadratically with the optimum inclusion rates being 3% to 6 % DM and 5 to 10 % DM respectively. Stock et al. (1990) observed a linear decrease in G:F whereas Kreikemeier et al. (1990) noticed a quadratic decrease for F:G.

Moreover according to Kreikemeier et al. (1990), diets containing 5% or 10 % roughage had a tendency of higher NEm and NEg than diets containing 0 or 15% roughage. According to May et al. (2007a), heifers fed 5% CS consumed less feed and were more efficient than heifers fed 15% CS when a portion of steam-flaked corn was replaced with 25% DGS in finishing diets.

When Mader et al. (1991) compared CS to AH in dry-rolled corn (DRC) or ground high moisture corn (GHMC) finishing diets, steers fed CS gained faster than those fed AH whereas a roughage source x grain type interaction was observed for DMI and G:F. Theurer et al. (1999) fed alfalfa, cottonseed hulls, and wheat straw to steers as the roughage source in three finishing diets. Steers fed alfalfa hay in steam flaked sorghum grain diets had lower DMI than steers fed diets containing alfalfa hay and cottonseed hulls or alfalfa and wheat straw. Although daily gains were not affected by roughage source, cattle fed alfalfa and wheat straw had a better feed efficiency.

It is clear that roughage source and level substantially affect DMI of cattle fed high-concentrate diets. However, reasons for increased DMI with changes in roughage level and source are not fully understood. In agreement to previous research (Kreikemeier et al., 1990, Stock et al., 1990 and May et al., 2007a), in our study CS and AH were fed at percentages that allow optimum performance; CS was fed at 10% of diet DM and AH was fed at 6% of DM. Changes in the fraction of dietary NDF supplied by roughage as levels and sources change seem to be associated with effects of roughage level and source on DMI. Galyean and Defoor (2003) suggest that effects of larger changes in roughage level (e.g., greater than 5% of DM) on DMI may reflect energy dilution, such that cattle increase DMI presumably in an attempt to maintain energy intake. Results from Defoor et al. (2002) suggest that roughage source with a higher NDF concentration has a greater roughage value; roughage value being the ability to promote NEg

intake in high-concentrate diets. According to NRC (1996) NDF of CS and AH are 46% and 43.9% respectively; NEm and NEg for AH are 1.31 and 0.74 Mcal/kg whereas for CS the values are 1.69 and 1.08 for NEm and NEg respectively. However, in our study NEm and NEg calculated based on animal performance for diets containing CS or AH were not different (P> 0.20). In our study CS and AH were assumed to be 50 and 100 % roughage respectively which might explain similarity of our roughage in terms of NEm and NEg, as well as similar ADG and G:F we obtained among cattle fed CS or AH.

Carcass Characteristics

There were no differences among treatment groups with regard to HCW, dressing percent, LM area, KPH, and subcutaneous fat over the 12th rib, but there was a roughage x DDGS level interaction (P = 0.02) for the incidence of liver abscesses, as well as for liver abscesses with a severity of A- (P = 0.03; Table 2-3). The highest incidence was found in cattle fed SFC_CS followed by those fed DDGS_AH; the lowest incidence occurring in cattle fed SFC_AH. Feeding 25% DDGS in replacement of SFC did not affect (P > 0.20) marbling score, but cattle fed corn silage tended (P = 0.10) to have greater marbling score than those fed alfalfa hay. Feeding DDGS did not affect USDA quality grades (P > 0.05) or USDA yield grades (P > 0.05; Table 2.4). Overall, heifers were relatively lean. The average yield grade for all treatment groups was near to 2.

Similar to our results, May et al. (2007b) found no differences among treatments for HCW, subcutaneous fat, and carcass quality grades, but unlike our results yield grades were not different when a portion of SFC was replaced with DGS. Likewise, according to Ham et al. (1994) steers fed rations where DRC was replaced by ethanol byproducts at 15, 25, or 40% (DM), had similar subcutaneous fat, quality grades, yield grades, and liver abscess scores.

Additionally, fat thickness, liver abscess scores, and quality grades were not affected by replacement of DRC with wet distiller's by product (Larson et al., 1993; Lodge. et al., 1997). On the other hand, Al-Suwaiegh et al. (2002) observed that hot carcass weight for steers fed wet corn or sorghum DG was heavier than steers fed the control diet. Hot carcass weight was similar between steers fed the wet corn or sorghum DG which is in agreement with the results of Depenbusch et al. (2007) where they observed no difference in 12^{th}-rib fat thickness and USDA yield grade when 25% corn WDGS was fed to yearling heifers as partial replacement of SFC. However, these authors did find a significant reduction in LM area, marbling score, and USDA Choice or better carcasses. Research by Daubert et al. (2005) showed that increasing sorghum-wet DGS from 0% to 40% linearly increased USDA yield grade while it decreased marbling score linearly. According to Owens and Gardner (2000), changes in yield grade may be attributable to less ruminal escape of dietary starch. Moreover, results from Vander Pol et al. (2006) suggested that a combination of flaked grains with distiller's by products may be deleterious to marbling deposition and yield grades.

In accordance with our results, Stock et al. (1990) noticed no roughage effect on quality grade and liver abscess scores. Similarly, Kreikemeier et al. (1990) found no roughage effect on carcass traits among treatments, but unlike our results, 55% to 71% of the livers were condemned because they did not feed Tylosin. Results from May et al. (2007b) indicated that heifers fed DDGS_CS 5% had a higher dressing percent over heifers fed SFC_CS 15% when a portion of SFC was replaced with DGS. Research by Bartle et al. (1994) suggests a quadratic decrease for marbling score, and percent choice as roughage level increases. HCW on the other hand decreases linearly as roughage inclusion rate increases. In our study cattle fed CS had only 3% greater ($P = 0.10$) marbling score than those fed AH. Owens and Gardener (2000) and Pingel

and Trenkle (2006) suggest that lower level of starch digestibility could affect marbling adipocytes deposition. We fed AH at 6% DM and CS at 10% DM assuming that the latter was 50:50 grain:roughage. We can speculate that although the overall performance was not affected by roughage source given its proportion in the entire diet, the grain portion of CS affect marbling score to some extent compared to AH ($P = 0.10$).

A roughage x DDGS level interaction ($P = 0.02$) for the incidence of liver abscesses, as well as for liver abscesses with a severity of A- ($P = 0.03$) was observed in our study. Heifers fed SFC_CS had the highest incidence (11.1%) while heifers fed SFC_AH had the lowest incidence (3.4%). Research from Mader et al.(1991) indicated that steers fed ground high moisture corn (HMC) diets with CS as roughage tended to have greater liver abscess scores than steers fed other diets. Many factors including amount, type, and physical characteristics of roughage affect the incidence of liver abscesses scores (Nagaraja and Chengappa, 1998). Generally, unlike our results, cattle fed dry hay tended to have a greater incidence of liver abscesses than cattle fed silage as roughage source (Mader et al., 1991; Mader, et al., 1993). The percentage of liver abscess incidence observed in our study is lower than the normal average ranging from 12 to 32% observed in most commercial feedlots (Brink et al., 1990).

Conclusions

The replacement of SFC with 25% of DDGS did not affect growth performance of finishing heifers. Heifers fed corn silage had greater DMI than those fed alfalfa hay but daily gains and feed efficiencies were not affected by roughage source. Substituting DDGS for steam-flaked corn resulted in similar carcass characteristics with only minor differences in quality and yield grades. Corn silage and alfalfa hay can be fed interchangeably in finishing diets when a

portion of SFC is replaced with DDGS without compromising growth performance or carcass quality.

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Table 2-1 Composition of experimental finishing diets (DM basis)

	Alfalfa hay		Corn silage	
Ingredients, (% DM)	0 % DDGS	25% DDGS	0 % DDGS	25% DDGS
Steam-flaked corn	82.8	59.8	76.6	54.9
Dried distiller's grains with solubles	--	24.3	-	24.0
Alfalfa hay	5.6	5.6	-	-
Corn steep liquor	6.0	6.1	6.0	6.0
Corn silage	--	--	11.0	11.0
Urea	1.3	--	1.2	--
Soybean meal	--	--	0.8	--
Limestone	1.7	1.7	1.7	1.6
Supplement[1]	2.6	2.5	2.7	2.5
Analyzed composition, (%)				
Dry matter	80.0	81.2	70.1	70.5
Crude protein	14.5	16.1	14.4	15.5
DIP	8.3	7.5	7.6	7.5
Ether extract	3.7	5.2	3.6	5.1
NDF	10.5	17.0	12.6	19.2
Starch availability,%[2]	48.1	48.1	48.1	48.1
Calcium	0.7	0.7	0.7	0.7
Phosphorus	0.3	0.5	0.5	0.5
Potassium	0.7	0.7	0.7	0.7

[1] Formulated to provide 300 mg/day monensin, 90 mg/day tylosin, 0.5 mg/day melengesterol-acetate, 2,200 IU/kg vitamin A, 0.3 % salt, 22 IU/kg vitamin E, 60 mg/kg Mn, 60 mg/kg Zn, 0.63 mg/kg I, 0.25 mg/kg Se, and 0.1 mg/kg Co.

[2] Starch availability was measured only for steam-flaked corn using the refractive index procedure.

Table 2-2 Growth performance of heifers fed finishing diets based on steam flaked corn containing alfalfa hay or corn silage with or without dried distiller's grains with solubles

	Alfalfa hay		Corn silage			P values		
Item	0% DDGS	25% DDGS	0% DDGS	25% DDGS	SEM	Roughage source	DDGS level	Roughage source x DDGS level
n	89	90	90	89	-	-	-	-
Days on feed	97	97	97	97	-	-	-	-
Initial BW, kg	353.6	353.6	353.6	353.3	13.0	0.89	0.95	0.61
Final BW, kg[a]	498.4	493.5	501.5	495.2	4.1	0.56	0.19	0.86
DMI, kg/d	8.02	7.83	8.33	8.09	0.23	0.05	0.14	0.88
ADG, kg/d	1.49	1.44	1.53	1.46	0.04	0.22	0.19	0.91
G:F	0.189	0.186	0.186	0.183	0.02	0.63	0.67	0.99
NE, Mcal/kg[b]								
Maintenance	2.66	2.64	2.62	2.60	0.02	0.77	0.58	0.97
Gain	1.93	1.91	1.89	1.87	0.18	0.76	0.57	0.98

[a] Final body weight calculated by dividing hot carcass weight by a common dressing of 63.5%

[b] Calculated based on animal performance using prediction equations from Beef NRC, 1984

Table 2-3 Carcass characteristics of heifers fed finishing diets based on steam-flaked corn containing alfalfa hay or corn silage with or without dried distiller's grains with solubles

	Alfalfa hay		Corn silage			P values		
Item	0% DDGS	25% DDGS	0% DDGS	25% DDGS	SEM	Roughage source	DDGS level	Roughage source x DDGS level
HCW, kg	316.4	313.4	318.5	314.4	2.64	0.60	0.21	0.82
Dressing, %	62.9	63.7	62.9	63.4	0.02	0.83	0.43	0.85
LM area, cm^2	82.8	83.7	83.8	82.3	1.29	0.78	0.77	0.25
Kidney, pelvic and heart fat, %	2.14	2.13	2.13	2.16	0.03	0.74	0.81	0.60
12^{th} rib fat, cm	1.27	1.24	1.22	1.14	0.08	0.29	0.53	0.79
Marbling score[a]	476	466	482	491	8.91	0.10	0.96	0.31
Liver Abscesses,%	3.4	7.9	11.1	5.6	2.00	0.20	0.82	0.02
A+	1.11	1.11	1.11	1.11	1.11	1.00	1.00	1.00
A_0	1.11	1.11	2.22	1.11	1.19	0.54	0.54	0.54
A-	1.19	5.72	7.78	3.41	1.98	0.27	0.96	0.03

[a] slight:400 to 499

Table 2-4 USDA yield grades and quality grades of heifers fed finishing diets based on steam flaked corn containing alfalfa hay or corn silage with or without dried distiller's grains

	Alfalfa hay		Corn silage			P- values		
Item	0% DDGS	25% DDGS	0% DDGS	25% DDGS	SEM	Roughage source	DDGS level	Roughage source x DDGS level
USDA Yield grade	2.31	2.22	2.31	2.31	0.11	0.68	0.67	0.67
Yield grade 1, %	12.6	22.9	11.0	14.8	4.97	0.29	0.20	0.57
Yield grade 2, %	50.5	36.9	48.9	41.7	5.60	0.75	0.04	0.50
Yield grade 3, %	30.1	34.7	37.8	41.3	5.84	0.22	0.48	0.94
Yield grade 4, %	5.7	5.5	2.3	2.2	2.25	0.16	0.63	1.00
Yield grade 5, %	1.1	0.0	0.0	0.0	0.56	0.33	0.33	0.33
USDA quality grade								
Prime, %	0.0	1.1	0.0	0.0	0.56	0.33	0.33	0.33
Choice, %	36.0	30.3	35.6	44.8	4.81	0.15	0.71	0.13
Select, %	59.5	61.8	63.3	48.5	4.39	0.30	0.17	0.07
Standard, %	4.5	6.8	1.1	6.7	4.61	0.12	0.11	0.54

CHAPTER 3 - Effects of roughage source and dried distiller's grains on ruminal fermentation and apparent total tract digestibility of finishing diets

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Abstract

Ruminal fermentation characteristics and diet digestibility were examined in cannulated Holstein steers (n=12; BW 487±18 kg) fed steam-flaked corn (SFC) finishing diets with 0 or 25% dried distiller's grains with solubles (DDGS), using alfalfa hay (AH) or corn silage (CS) as roughage sources. The study was a randomized incomplete block design with a 2 × 2 factorial arrangement of treatments. Factors were DDGS level (0 or 25% of DM) and roughage source (6% AH or 10% CS, DM basis). The study was conducted in two periods, each consisting of a 14-d adaptation phase and a 3-d collection phase, with 3 animals assigned to each treatment in each period. Ruminal content samples and fecal samples were collected at 2-h intervals post-feeding over a period of 24 h. Ruminal content samples were used to determine ruminal pH and ruminal concentrations of ammonia, VFA, and lactate. Fecal samples were pooled within animal and period, and used to determine total fecal output and apparent total tract digestibility of DM, OM, NDF, CP, starch, and ether extract. One animal was removed from analyses due to illness during the study. Ruminal pH for all treatments was below 5.8 for 14 h after feeding. Steers fed 25% DDGS had lower A:P ratio ($P < 0.05$) but higher ruminal lactate concentration ($P < 0.05$) than cattle fed 0% DDGS. Feeding 25% DDGS decreased ruminal ammonia concentration ($P < 0.05$) and yielded lower digestion of DM and OM ($P < 0.05$), but percent NDF digestion was similar among treatments ($P > 0.10$). However cattle fed DDGS had greater ($P < 0.01$) fecal NDF compared to those fed diets without DDGS. The decrease in digestibility was not only largely attributable to a depression in digestion of CP ($P = 0.02$) and NDF, but also, to a lesser extent, poorer starch digestion ($P = 0.01$) when DDGS was fed. When DDGS replace a portion of corn and DIP source such as urea in SFC diets, ruminal availability of protein may be a limiting factor for bacterial activity and subsequent fermentation, regardless of roughage source.

Introduction

Rapid expansion of fuel ethanol production in the High Plains, where feedlots commonly steam flake feed grains prior to feeding, has popularized substitution of a portion of steam-flaked corn with distiller's grains with solubles (DGS). Most of the starch and degradable protein of corn are degraded during the process of fermenting the grain into ethanol. The remaining components are concentrated in DGS, which is rich in NDF, ruminal undegradable protein, and fat (NRC, 1996). Different extents of diet digestion have been reported due to feeding different levels of DGS. In a study by Depenbuch et al. (2007), cattle fed traditional dried distiller's grains with solubles (DDGS) or de-germed distiller's grains had lower diet digestion compared to their cohorts fed diets without traditional or de-germed distiller's grains. Moreover, heifers fed DDGS with alfalfa hay in steam-flaked corn diets had lower performance than the control group (May et al., 2007a), but they had performance similar to that of the control group when corn silage was fed as roughage source (May et al., 2007b).

The activity of ruminal microorganisms and diet utilization depend on ruminal pH, quantity, degradability, and quality of energy and protein source (Matras et al., 1991). Ruminal pH normally is observed below 6 in cattle fed flaked grain finishing diets (Sindt et al., 2006). We speculated that low ruminal pH may impede digestion of distiller's grains fed in steam-flaked corn diets, due to their high NDF content. Additionally, the effects of roughage sources on DMI seem to be associated with differences in ruminal fermentation and digesta kinetics due to their different physical and chemical characteristics. Thus, the purpose of this study was to examine ruminal fermentation characteristics and diet digestibility when steam-flaked corn finishing diets are fed with 0 or 25% DDGS, using alfalfa hay or corn silage as roughage sources.

Materials and Methods

Procedures followed in the present study were approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2535.

Ruminal fermentation characteristics and diet digestibility were examined in cannulated Holstein steers (n=12; BW 487±18 kg). The study was a randomized incomplete block design with a 2 × 2 factorial arrangement of treatments. Factors were level of dried distiller's grains with solubles (DDGS, 0 or 25% DM) and roughage source (alfalfa hay, AH or corn silage, CS). Experimental diets were based on steam-flaked corn and contained 0% DDGS with 6% AH (SFC_AH), 0% DDGS with 10% CS (SFC_CS), 25% DDGS with 6% AH (DDGS_AH), or 25% DDGS with 10% CS (DDGS_CS). The diet composition is further described in Table 3-1. Steers were housed in individual slatted floor pens measuring 1.5 m x 3 m each. Pens were equipped with individual feed bunks and water fountains that allowed *ad libitum* access to feed and clean water. Steers were given a unique ear tag identification number, randomly assigned to a dietary treatment, and individually weighed at the beginning and the end of each feeding/sampling period. They also were observed daily for clinical signs of digestive and (or) metabolic disorders or other diseases throughout the study. Diets were mixed once daily immediately prior to feeding for *ad libitum* intake and the weights of fresh feed provided and feed removed were recorded. Feedstuffs were sampled weekly and analyzed for DM, protein content, particle size distribution, and starch availability.

The study consisted of feeding periods, with three animals per treatment each feeding period (6 observations per treatment). However, one animal became ill and was removed from both periods of the study due to poor intake and excessive body weight loss. To ensure a smooth dietary transition between the two feeding periods, there was a 3-day transition period, followed

by a 14-day acclimatation to experimental diets. Ruminal contents samples and fecal samples were collected over a period of 3 days.

Chromic oxide was used as an indigestible marker to estimate total fecal output. Chromic oxide boluses (10 g) were ruminally dosed daily before feeding for 7 days prior to sampling. Rumen fluid (RF) samples and fecal samples were taken 4 times a day at 6-h intervals, with the sampling time advanced by 2 h each day. Thus, within the 3-day sample period, samples were collected at 2-h intervals post-feeding. Composite fecal samples from each steer were used to determine apparent total tract digestibility of DM, OM, NDF, CP, starch, and crude fat for each period. Individual RF samples from each steer were used to measure ruminal pH, VFA profile, lactate, and ammonia concentrations.

Fecal samples for each steer were composited within period. Diet samples and orts were dried in a forced air oven at 55° C for 24 h, air equilibrated overnight, and weighed to determine partial DM (Understander et al., 1993). Dried samples were then ground in a Wiley mill (Thomas Scientific, Swedesboro, NJ) through a 1-mm screen and stored in plastic containers at room temperature for subsequent analyses. Based on the procedure described by Understander et al. (1993), a portion of the ground samples were dried in a forced air oven at 105° C overnight, and combusted at 450° C for 8 h to determine laboratory DM and OM, respectively. Chromium concentrations in fecal samples were measured using an atomic absorption spectrophotometer with an acetylene/air flame (Perkin Elmer 3110, Norwalk, CT) as described by Williams et al. (1962). Starch content was determined according to Herrera-Saldana and Huber (1989) with free glucose (Gochman and Schimitz, 1972) by using a Technicon Autoanalyzer III (SEAL Analytical, Mequon, WI), and NDF was analyzed using an ANKOM fiber analyzer (ANKOM technology Corp., Fairport, NY). To determine crude protein (CP = 6.25 X N), N was measured

by the AOAC (1995) official method 990.03 using a LECO FP–2000 nitrogen analyzer (LECO Corp., St Joseph, MI), and ether extract was analyzed according to the AOAC official method 920.39 (AOAC, 1995).

Total tract digestibilities of nutrients were obtained by dividing the difference between nutrient intake of each steer per period and its fecal output (FO) by nutrient intake. Fecal output was determined by dividing Cr by the concentration of chromium in feces.

Ruminal fluid (RF) samples were strained through eight layers of cheesecloth and pH was immediately recorded. Four mL of strained RF were combined with 1 mL of 25% (w/v) metaphosphoric acid to precipitate proteins. Thereafter, the acidified RF samples were immediately frozen at -20°C for further analyses. After thawing, the acidified RF was centrifuged at 30,000 × g for 20 min, and 1 mL of the supernatant was analyzed for VFA and lactate by gas chromatography (Hewlett-Packard 5890A, Palo Alto, CA; 2 m x 2 mm column; Supelco Carbopack B-DA 80/120 4% CW 20 m column packing, Bellefonte, PA) with He as the carrier gas, a flow rate of 24 mL/min, and a column temperature of 175°C. Total VFA concentration was computed as the sum of individual amounts of different VFA at each sampling time. A/P ratio was computed by dividing the concentration of acetate by the concentration of propionate. Ruminal ammonia concentration was determined using the Technicon Autoanalyzer (SEAL Analytical, Mequon, WI) according to Broderick and Kang (1980).

The value of protein fed to cattle is influenced by the extent to which it is degraded in the rumen. Thus, protein degradation of DDGS was determined by *in vitro* ammonia and total amino acid (TAA) release assay as described by Broderick (1987). Whole ruminal contents were obtained from a ruminally cannulated steer fed a SFC-based finishing diet with 25% DDGS (DM basis.) Ruminal contents were then strained through two layers of cheesecloth. To extract some

of the particle-associated organisms, the solid residue was washed four times with an amount of McDougall's buffer equal to the original amount of strained rumen fluid (SRF). The washout and the SRF were mixed, filtered through eight layers of cheesecloth, and bubbled with carbon dioxide to purge air. The strained rumen *in vitro* inoculum was prepared following the method described by Craig et al. (1984). Hydrazine sulfate (HS), cloramphenicol (CAP), and 2-mercaptoethanol were used as inhibitors of N assimilation, and maltose was used as an energy source. The composition of *in vitro* inoculum and reagent concentrations in the final medium are displayed in Table 3-5. The substrates were DDGS, soybean meal (SBM), and sodium caseinate, with SBM and sodium caseinate serving as controls. DDGS and SBM were finely ground with a cyclone mill (Udy Corporation, Fort Collins, CO). Finely ground samples containing 3 mg N were weighed into plastic incubation tubes, and wetted with 8 mL of McDougall's buffer for about 1 h. Blank tubes (containing inoculum and ruminal fluid but without substrate) also were included. Tubes were covered with stoppers and placed into a shaking water bath in an incubation room where the temperature was set at 39°C. The prepared mixture of SRF and extract of buffer were warmed as well, and appropriate reagents were added to the inoculum 5 min prior to starting incubation. After mixing, 16 mL of the inoculum plus reagents were added to all tubes and incubated for 4 h. There were 3 replicates per sample and blank per time point. Incubation was halted by adding 2 mL of 65 % (w/v) trichloroacetate (TCA) to each tube and subsequently placing into ice for 30 min to cool. One mL of each sample was then transferred into 12 × 75 mm tubes and centrifuged at 15,300 × g at 4°C for 15 min. The supernatant was kept at 4°C until analysis for ammonia and TAA. Recoveries of ammonia and TAA-N, after each h were computed using the following equations:

Ammonia recovery (%) = (NH_3-N/ NH_3-N added) × 100,

and $$\text{N recovery (\%)} = (NH_3\text{-N} + \text{TAA-N/added N}) \times 100$$

In this formula, TAA-N was computed from the TAA content of acid hydrolysates of each feedstuff. The proportion degraded (PD) for each sample for each time period was calculated using the following formula:

$$PD = \{\text{mg } NH_3\text{-N } (\text{at } t) + [(\mu\text{mol TAA } (\text{at } t))/(\mu\text{mol TAA/mgN})]\}/\text{mg added N}$$

Statistical analyses

Apparent total tract digestibilities were analyzed using the Mixed Procedure of SAS version 9.1 (SAS Inst., Cary, NC). Animal was the experimental unit, and period was included as a random effect. The model statement included effects of DDGS level, roughage source, and the interaction between DDGS level and roughage source. Repeated measures analyses were performed for ruminal pH, ruminal concentrations of ammonia, VFA and lactate using the Mixed models procedure also. The model statement included DDGS level, roughage source, time post feeding, interaction between DDGS level and roughage source, interaction between DDGS level and time post-feeding, interaction between roughage source and time post-feeding, and interaction between DDGS level, roughage source, and time post-feeding. Treatment means were determined by using LSMEANS option, and they were separated using F-test protected LSD ($P \leq 0.05$).

Results and Discussion

Treatment effects on intake and fecal excretion by cannulated Holstein steers are summarized in Table 3-2. There was an interaction ($P < 0.01$) between levels of DDGS and roughage source with respect to DMI and OMI. Intakes of DM and OM were lower when 25% DDGS was fed with CS as roughage source compared to other treatment groups. This interaction

may reflect a particular case rather than a reality because an interaction was not observed in our performance study in which cattle were fed diets similar to those fed in this study. In the performance study cattle fed CS had greater (P = 0.05) DMI compared to those fed AH. Depenbusch et al. (2007) observed that including 13% DDGS or de-germed DDGS in SFC-based diets with AH as a roughage source did not affect DMI or OMI, but May (2007) reported reduced intake of DM and OM by feeding 25% DDGS in SFC-based diets using CS as a roughage source.

A DDGS level by roughage source interaction was observed with regard to NDF intake (P < 0.01). The highest NDF intake was observed when cattle were fed 25% DDGS with AH as a roughage source, the lowest NDF intake occurred when cattle were fed DDGS with CS as roughage source presumably because of lower DMI observed in DDGS_CS. Despite the interaction observed for NDF intake, steers fed 25% DDGS had greater (P < 0.01) NDF intake than steers fed no DDGS. Similar to our results, May (2007) reported an increase in NDF intake when replacing a portion of SFC with 25% DDGS. Depenbusch et al. (2007) also observed increased NDF intake when they fed 13% DDGS or de-germed DDGS in SFC diets. In our study, the analyzed NDF values of DDGS, AH, CS, and SFC were 35.3; 54.7, 52.4, and 9.0%, respectively. Based on these values, more NDF intake would be expected when replacing a portion of SFC with DDGS.

There was an interaction (P = 0.01) between DDGS and roughage source with respect to starch intake. Steers fed diets without DDGS consumed more starch when CS was fed but steers fed diets with DDGS had lowest starch intake when AH was fed. This observation was not surprising because CS was estimated to contain 50:50 grain:roughage whereas both DDGS and AH are poor in starch content. On the other hand, steers fed diets containing DDGS had greater

($P < 0.05$) intake of ether extract compared to steers fed no DDGS. Similar to our results, May (2007) observed a decline in starch intake when DDGS was included in the diet, but unlike our results, he also observed a decline in intake of ether extract when 25% DDGS were fed compared to 0% DDGS. In the trial by May (2007), diets were balanced to include similar ether extract content across treatments but this was not the case in our study. Ether extract content of DDGS fed in our trial was 10.1% vs. only 4.3% for SFC; hence, a greater intake of ether extract in our diets containing DDGS compared to the diets without DDGS was expected. On the contrary, most of the starch is fermented during ethanol production, resulting in a very small amount of starch contained in DDGS, which explains the magnitude of the differences in starch intake between the diets containing DDGS and the controls. Feeding DDGS did not affect ($P > 0.05$) CP intake. This observation was expected because the diets were formulated to be isonitrogenous.

Dietary effects on ruminal pH are presented in Figure 3-1. Ruminal pH reached the lowest point 4 h post-feeding (5.07; 5.15; 5.11; and 5.14 for DDGS_AH; DDGS_CS; SFC_AH; and SFC_CS, respectively). A 3-way interaction ($P < 0.01$) occurred between the levels of DDGS, roughage source and time post-feeding. Although ruminal pH for all dietary treatments was below 5.8 until 14 h post-feeding, steers fed 25 % DDGS had lower pH when AH was fed, but they had the highest pH when CS was used as a roughage source from 12 to 22 hour after feeding. The average pH over a 24-h period was 5.31, 5.72, 5.49, and 5.56, respectively for DDGS_AH, DDGS_CS, SFC_AH, and SFC_CS. Intakes for cattle fed DDGS_CS were much lower than other treatments and this is likely the major factor that explains higher pH observed in cattle fed DDGS using CS as a roughage source. Similar to our results, in a study by May (2007), steers fed 25% DDGS steam-flaked corn diets, with corn silage as roughage source, had lower

ruminal pH more than 12 h after feeding compared to their counterparts fed diets without DDGS. Likewise, research by Corrigan et al. (2008) indicated that cattle fed 40% WDGS (DM basis) in diets consisting of dry-rolled corn, high moisture corn, or steam-flaked corn with AH as a roughage, had lower ruminal pH than the control group fed diets without WDGS. Ruminal pH of feedlot cattle is usually below pH 6.0 (Zinn et al., 1995; Corona et al., 2006; Sindt et al., 2006). Hoover (1986) reported that optimum fiber digestion is obtained at ruminal pH between 6.2 and 6.8. In our study, those pH values were attained only more than 14 h after feeding regardless of the level of DDGS. Feeding DDGS presumably would increase ruminal pH in high concentrate diets since starch is extracted during fermentation process and the fiber content is increased (Klopfenstein et al., 2007). However, the physical effectiveness of NDF in distiller's grains to stimulate rumination is limited because of small particle size (Bhatti and Firkins, 1995). Hence, lack of fiber effect may explain low ruminal pH observed in cattle fed distiller's grains. Additionally, the optimal pH for ruminal proteolytic bacteria ranges from 5.5 and 7.0 with more deleterious effects at the lower end of ruminal pH (Bach et al., 2005). Because the bulk of ruminal digesta is digested during the first 6 h post-feeding when pH was below 5.5 in this study, it is probable that ruminal pH was a limiting factor for bacterial growth and subsequent fermentation. The rise in ruminal pH after 16 h post-feeding likely indicates the end of VFA production due to minimal presence of fermentable organic matter.

When analyzing total VFA concentrations, there were no 3-way interactions ($P > 0.10$) between the main effects and time post-feeding (Figure 3-8). However, there was an interaction ($P < 0.05$) between DDGS level and roughage source. Total VFA concentration was lowest when 25% DDGS was fed using CS as roughage, but it was not affected by DDGS level when AH was fed. As expected, there also was an effect of time after feeding ($P < 0.05$). VFA concentration

dropped during the second half of a 24-h measurement period, indicating a decrease of fermentable organic matter in the rumen. The average total VFA concentrations were, respectively, 118.4, 105.0, 120.2, and 125.5 mM for DDGS_AH, DDGS_CS, SFC_AH, and SFC_CS. As mentioned previously, the low VFA concentrations observed in cattle fed DDGS_CS is likely intake driven because these cattle had lower intake compared to other groups. No interactions between effects of time after feeding, DDGS level, or roughage source were observed ($P > 0.10$) with respect to ruminal acetate concentrations (Figure 3-3). Feeding 25% DDGS resulted in lower ($P = 0.01$) ruminal acetate concentrations in comparison to feeding diets without DDGS. As expected, time post-feeding influenced acetate concentration in the rumen ($P < 0.01$). For all dietary treatments, peak acetate concentrations were reached 6 h after feeding (54.4; 68.7; 66.2; and 74.2 mM for DDGS_AH; DDGS_CS; SFC_AH; and SFC_CS respectively). On average, the concentrations of acetate in the rumen were 45.5, 47.3, 50.4, and 56.4 mM for DDGS_AH, DDGS_CS, SFC_AH, and SFC_CS, respectively.

No 3-way interactions ($P > 0.10$) were observed with respect to ruminal propionate concentration (Figure 3-4). However, there was an interaction ($P < 0.05$) between DDGS level and roughage source. Propionate concentration was the lowest when 25% DDGS was fed using CS as roughage source but it was not affected by DDGS level when AH was used as a roughage source. The average propionate concentrations were 50.7, 42.2, 49.5, and 49.4 mM for DDGS_AH, DDGS_CS, SFC_AH, and SCF_CS, respectively. There were no interactions ($P > 0.10$) for A/P ratio (Figure 3-7). Steers fed 25% DDGS had 12.1% lower ($P < 0.01$) A/P ratio than their counterparts fed 0 % DDGS. There was no 3–way interaction ($P > 0.10$) for ruminal butyrate concentration (Figure 3-5), but there was an interaction ($P < 0.01$) between DDGS level and roughage source. Cattle fed 25% DDGS had the highest ($P < 0.05$) butyrate concentration

when AH was used as a roughage source but they had the lowest butyrate concentration when CS was fed. The average butyrate concentrations were 15.5; 9.0; 14.5; and 13.3 mM for DDGS_AH; DDGS_CS; SFC_AH; and SFC_CS, respectively.

Similar to our results, May (2007) reported that feeding 25% DDGS with CS in SFC-based diets decreased ruminal concentrations of total VFA, acetate and propionate. On the other hand, in a study by Ham et al. (1994), steers fed 40% WDGS (DM basis) with 10% DM of a 50:50 mixture of CS:AH in dry-rolled corn diets had ruminal concentrations of total VFA, acetate, and propionate similar to their counterparts fed diets without WDGS. Peter et al. (2000) evaluated the use of DDGS with DRC in beef diets. In their study, contrary to our results, ruminal acetate concentration increased in cattle fed DDGS, and butyrate concentration increased with inclusion of DDGS in the diets which is similar to our results.

Ruminal pH is lower in cattle fed SFC compared to those fed DRC (May, 2007). Because distiller's grains is rich in nonforage fiber content, it was presumably digested more in DRC-based diets because of higher ruminal pH at which fibrolytic activity is active; hence the difference between results of this study and results by Ham et al. (1994) and Peter et al. (2000).

The concentrations of branched-chain VFA and valerate are summarized in Table 3-4. No interactions between the main effects were observed ($P > 0.10$) for isobutyrate and isovalerate. Feeding 25% DDGS did not affect isobutyrate concentration ($P > 0.10$), but resulted in 53% less isovalerate ($P < 0.01$) in comparison to not feeding DDGS. There was an interaction ($P < 0.05$) between DDGS level and roughage source with regard to valerate concentration. Ruminal valerate concentration was the highest when 25% DDGS was fed with AH as roughage but it was lowest when 0% DDGS was fed using CS as a roughage source.

Previous research indicated that feeding 25% DDGS (DM basis) in SFC diets (May, 2007), or feeding 40% DDGS (DM basis) in DRC-based diets (Ham et al., 1994), did not affect the concentrations of isobutyrate, isovalerate, and valerate. However, feeding 40% WDGS (DM basis) in DRC diets increased ruminal concentrations of isovalerate and valerate but had no effect on isobutyrate concentration (Ham et al., 1994). May (2007) fed CS as a roughage source, Ham et al. (1994) used a 50:50 mixture of AH and CS whereas we fed AH or CS as roughage sources. Branched-chain VFA are products of degradation of branched amino acids, but they are also, together with valerate, growth factors primarily metabolized in the rumen by fiber-digesting bacteria in the rumen (Nagaraja, personal communication). The level of their ruminal concentrations may thus indicate either the level of protein degradation or fiber digestion in the rumen.

There was no interaction ($P > 0.10$) between the main effects with regard to ruminal lactate concentration (Figure 3-6), but there was an interaction between time post-feeding and DDGS level ($P < 0.05$). Cattle fed DDGS had the highest lactate concentration during the first 8 h when compared to cattle not fed DDGS. This was followed by a sharp decline in concentration for the remainder of the 24 h digestion period. The average lactate concentrations for the main effect of DDGS level were 1.58 mM and 1.01mM for 25% DDGS and 0% DDGS, respectively. May (2007) also reported increased ruminal lactate concentrations when cattle fed diets with 25% DDGS were compared to those fed diets without DDGS. Because most of starch is removed during ethanol production, lower lactate concentrations were expected in steers fed diets with DDGS. In a study by Fron et al. (1996), cattle fed corn condensed distiller's solubles (CCDS) in DRC-based diets had higher ruminal lactate concentration than their counterparts fed diets without CCDS. It is likely that solubles blended back to distiller's grains contributed to higher

lactate concentrations and low ruminal pH observed in cattle fed diets with DDGS in our study. Although ruminal pH was low the majority of a 24-h period across treatments, higher ruminal lactate in cattle fed DDGS did not affect butyrate concentrations. However, it is possible that some of lactate was metabolized to propionate, hence higher propionate concentrations relative to acetate concentrations resulting in lower A:P ratio observed in cattle fed diets containing DDGS.

Changes over time in ruminal concentration of ammonia are presented in Figure 3-2. There was a tendency ($P = 0.06$) for an interaction between time post-feeding, DDGS level and roughage source. An interaction ($P < 0.05$) between DDGS level and roughage source was also observed. The highest ammonia concentration was observed when 0% DDGS was fed using AH as a roughage source but it was not affected by DDGS level when CS was fed as a roughage source. The average ruminal ammonia concentrations were 3.8, 3.5, 8.0, and 4.9 mM for DDGS_AH, DDGS_CS, SFC_AH, and SFC_CS, respectively. There was also an interaction ($P < 0.05$) between DDGS level and time post-feeding with respect to ammonia concentrations. Ruminal ammonia concentrations were lower in steers fed diets with 25% DDGS compared to those fed 0% DDGS averaged over the entire 24-h period, but concentrations in the latter dropped after 6 h post-feeding.

In accordance with our observations, Santos et al. (1984), Ham et al. (1994), and May (2007) observed decreases in ruminal ammonia concentration when feeding distiller's grains. Ammonia is a byproduct of protein degradation in rumen, and it is a source of nonprotein N necessary for ruminal microbial protein synthesis (Bach et al., 2005) which represents 50-80% of total absorbable protein in ruminants (Storm and Ørskov, 1983). Satter and Slyter (1974) suggested that ruminal ammonia concentration less than 2.94 mM hinders maximum microbial

protein production. In our study, ruminal ammonia concentrations of diets containing DDGS were lower than 2.94 mM for the first 10 h after feeding. The results of the ammonia release assay we conducted to determine the available DIP fraction of our DDGS revealed DDGS to contain 50% DIP (data not shown). Not only DDGS, which is only 50% degradable, replaced a portion of corn in our experimental diets, but also it replaced urea which is 100% degradable (NRC, 1996). It is conceivable that by feeding DDGS as a protein source in place of urea, nitrogen becomes limiting in the rumen. As a result, nitrogen assimilation and subsequent fermentation might be limited, yielding lower digestibility due to suboptimal microbial activity. Additionally, there might have been some positive effects of amylases on protein degradation which may explain lower ammonia concentrations observed in cattle fed diets with 25% DDGS as opposed to their counterparts fed diets without DDGS. According to Assoumani et al. (1991), addition of amylase increased total ruminal protein degradation of cereal grains between 6 and 20% units. Therefore, it is possible that replacing a portion of corn with DDGS reduces these positive effects of amylases on protein degradation, hence low ruminal ammonia concentration observed in steers fed diets with DDGS.

There was no interaction ($P > 0.10$) on apparent total tract digestibility of all nutrients investigated (Table 3-3). Feeding 25% DDGS resulted in lower ($P < 0.05$) apparent total tract digestibility of DM, OM, starch, and CP but had no effect on digestibility of NDF ($P > 0.10$) and ether extract ($P > 0.10$). Consequently, steers fed 25% DDGS excreted more ($P < 0.05$) DM, OM, NDF, and CP compared steers fed 0% DDGS. Similar to our results, Depenbusch et al. (2007) observed a decrease in total tract digestibility of DM and OM when they replaced a portion of SFC with 13% of DDGS or de-germed DDGS in the diets. Likewise, May (2007) observed a tendency to decrease DMD, OMD due to feeding 25% DDGS in either SFC or DRC-

based diets. On the other hand, research by Vander Pol et al. (2007) found no differences in digestibility between cattle fed DRC with wet distiller's grains, and DRC without wet distiller's grains.

We hypothesized that feeding DDGS in steam-flaked corn-based diets would have low fiber digestion due to low ruminal pH. Although percentage of fiber (NDF) digested was similar for cattle fed diets with or without DDGS, cattle fed diets with DDGS had greater NDF intake ($P < 0.01$) and greater NDF excretion ($P < 0.01$) compared to their cohorts fed diets without DDGS. Moreover, feeding 25% DDGS resulted in lower digestion of DM and OM compared to feeding diets without DDGS. This decrease seems to be attributable not only to greater NDF intake ($P < 0.01$), but also to a depression in digestion of CP ($P < 0.05$), and to a lesser extent, poorer starch digestion ($P < 0.05$) when DDGS replace a portion of the steam-flaked corn and urea. Additionally, ruminal ammonia concentrations were lower in steers fed diets containing 25% DDGS compared to those fed 0% DDGS. Although experimental diets were formulated to be isonitrogenous, DIP content was lower in diets with DDGS (Table 3.1). According to Klopfenstein et al. (2007), much of the protein in distiller's solubles is yeast cells which have been heated during distillation and concentration. Yeast concentrations often reach 150 million cells per cubic centimeter in mashes after 26 hours of fermentation (Hatch, 1995). Heat denatured yeast render distiller's solubles resistant to lyses and microbial degradation (Bruning and Yokoyama, 1988). Research by Herold (1999) suggested that only 20% of condensed distiller's solubles from the wet milling are degradable in the rumen. It is thus conceivable that replacing a portion of steam-flaked corn and urea with DDGS limits nitrogen availability, which may reduce digestibility due to suboptimal rumen microbial activity. It also is possible that low ruminal pH may depress activity of proteolytic bacteria. Research by Nuget and Mangan (1981)

indicated proteolyisis to be the rate-limiting step and, therefore, key in controlling protein degradation. The optimal pH of ruminal proteolytic enzymes varies between 5.5 and 7.0 (Bach et al., 2005). Thus, it is likely that, compared to digestion of other nutrients except NDF, protein degradation was low across all treatments due to low ruminal pH observed**.** When DDGS is added to steam-flaked corn diets at the expense of corn and urea, ruminal availability of protein may be the limiting factor for bacteria growth and subsequent fermentation.

Feeding AH increased ($P < 0.05$) intakes of CP, and ether extract compared to feeding CS. Cattle fed AH had greater digestibility of starch ($P = 0.02$) and CP ($P = 0.03$) than cattle fed CS. As discussed previously lower DMI observed in cattle fed DDGS_CS compared to the other treatments may be the reason of these differences rather than differences between AH and CS per se. Feeding CS resulted in 7.5% higher ($P = 0.01$) acetate concentration and 17.9% higher ($P < 0.01$) A/P ratio compared to feeding AH but roughage source did not affect ($P > 0.10$) lactate concentration. Steers fed CS had higher ($P < 0.01$) concentrations of isovalerate (40.3%), and isobutyrate (15.8%) compared to their cohorts fed diets with AH.

In a study by Poore et al. (1990), total tract digestibility of NDF was not altered as concentrate increased from 30 to 90%. In this study, the roughage source was a 50:50 mixture of wheat straw and alfalfa hay. According to Kreikemeier et al. (1990), adding AH from 5 to 15% in increments of 5% to a steam-rolled wheat diet increased the rate of starch digestion.

In our study, although some differences were observed in ruminal fermentation end products due to roughage sources, these differences were more likely intake driven as discussed previously. Additionally, roughage source did not affect total tract digestibility of any of the nutrients studied. This suggests that AH and CS has similar feeding value when fed in SFC-based finishing diets.

Conclusions

Partial replacement of steam-flaked corn with dried distiller's grains alters ruminal fermentation and diet digestibility. Feeding DDGS as partial replacement of steam-flaked corn and urea resulted in great NDF intake and excretion, and low ruminal ammonia concentrations. Digestion of NDF may have been inhibited at low ruminal pH observed whereas substituting urea with DDGS could have limited ruminal bacteria growth and fermentation of rumen digesta which resulted in an overall reduced total tract digestion of almost all nutrients by cattle fed 25% DDGS compared to their counterparts fed 0% DDGS. Feeding DDGS at moderate levels in SFC-based diets may require not only strategies to increase ruminal pH to ensure adequate NDF digestion, but also additional DIP supplementation to ensure adequate available nitrogen for bacterial growth and subsequent digestion of dietary organic matter.

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Table 3-1 Composition of experimental finishing diets based on steam–flaked corn containing 0 or 25% dried distiller's grains with solubles (DM basis) using alfalfa hay or corn silage as the roughage sources fed to cannulated Holstein steers

	Alfalfa hay		Corn silage	
Ingredients, (% DM)	0 % DDGS	25% DDGS	0 % DDGS	25% DDGS
Steam flaked corn	82.8	59.8	76.6	54.9
Dried distiller's grains with solubles	--	24.3	--	24.0
Alfalfa hay	5.6	5.6	--	--
Corn steep liquor	6.0	6.1	6.0	6.0
Corn silage	--	--	11.0	11.0
Urea	1.3	--	1.2	--
Soybean meal	--	--	0.8	--
Limestone	1.7	1.7	1.7	1.6
Supplement[1]	2.6	2.5	2.7	2.5
Analyzed composition (%)				
Dry matter	80.0	81.2	70.1	70.5
Crude protein	14.5	16.1	14.4	15.5
DIP	8.3	7.5	7.6	7.5
Ether extract	3.7	5.2	3.6	5.1
NDF	10.5	17.0	12.6	19.2
Calcium	0.7	0.7	0.7	0.7
Phosphorus	0.3	0.5	0.5	0.5
Potassium	0.7	0.7	0.7	0.7

[1] Formulated to provide 300 mg/day monensin, 90 mg/day tylosin, 2,200 IU/kg vitamin A, 0.3 % salt, 22 IU/kg vitamin E, 60 mg/kg Mn, 60 mg/kg Zn, 0.63 mg/kg I, 0.25 mg/kg Se, and 0.1 mg/kg Co

Table 3-2 Intake and fecal excretion by cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources

	Alfalfa hay		Corn silage			P values		
	0% DDGS	25% DDGS	0% DDGS	25% DDGS	SEM[a]	Roughage source	DDGS level	Roughage x DDGS level
n	5	5	6	6				
Intake, kg/d								
DM	8.27	8.55	8.55	7.37	0.32	0.02	0.02	< 0.01
OM	8.15	8.29	8.37	7.19	0.31	0.02	0.01	< 0.01
Starch	5.71	4.46	5.83	3.76	0.21	0.02	< 0.01	0.01
NDF	0.89	1.46	0.97	1.33	0.04	0.28	< 0.01	< 0.01
CP	1.30	1.36	1.18	1.14	0.05	< 0.01	0.48	0.09
Ether extract	0.285	0.418	0.246	0.373	0.02	0.05	< 0.01	0.89
Fecal output, kg/d								
DM	1.27	1.99	1.57	1.85	0.11	0.43	0.01	0.04
OM	1.15	1.78	1.41	1.69	0.10	0.68	0.01	0.08
Starch	0.08	0.09	0.12	0.13	0.01	0.07	0.27	0.27
NDF	0.53	0.92	0.64	0.88	0.06	0.15	< 0.01	0.12
CP	0.25	0.38	0.31	0.36	0.02	0.34	0.01	0.09
Ether extract	0.041	0.055	0.036	0.044	0.004	0.27	0.03	0.52

[a]: When observations are missing, larger SEM is presented

Table 3-3 Digestion characteristics of cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources

	Alfalfa hay		Corn silage			P values		
Item	0% DDGS	25% DDGS	0% DDGS	25% DDGS	SEM[a]	Roughage source	DDGS level	Roughage x DDGS level
n	5	5	6	6				
Apparent total tract digestion, kg/d								
DM	7.00	6.56	6.98	5.52	0.33	0.03	< 0.01	0.06
OM	7.00	6.50	6.96	5.50	0.35	0.07	< 0.01	0.04
Starch	5.64	4.37	5.71	3.63	0.20	0.01	< 0.01	0.01
NDF	0.36	0.54	0.33	0.45	0.07	0.48	0.10	0.69
CP	1.05	0.98	0.87	0.78	0.05	< 0.01	0.07	0.84
Ether extract	0.244	0.363	0.210	0.329	0.020	0.13	< 0.01	0.99
Apparent total tract digestibility, %								
DM	83.5	76.9	81.6	76.1	1.43	0.41	0.01	0.73
OM	84.7	78.4	83.7	77.8	1.55	0.63	0.01	0.91
Starch	98.7	97.7	98.0	96.8	0.31	0.07	0.02	0.77
NDF	39.8	37.8	31.1	32.9	6.22	0.35	0.99	0.79
CP	79.8	72.8	74.6	69.8	1.96	0.10	0.03	0.61
Ether extract	84.8	86.8	85.2	87.7	1.31	0.66	0.17	0.85

[a]: When observations are missing, larger SEM is presented

Table 3-4 Minor VFA concentrations in cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources

	Alfalfa hay		Corn silage			P values		
Item	0% DDGS	25% DDGS	0% DDGS	25% DDGS	SEM[a]	Roughage source	DDGS level	Roughage x DDGS level
n	5	5	6	6				
Isobutyrate, mM	0.64	0.69	0.79	0.79	0.10	< 0.01	0.53	0.45
Isovalerate, mM	2.16	0.68	3.02	1.75	0.41	< 0.01	< 0.01	0.62
Valerate, mM	3.51	5.31	2.84	3.56	0.67	< 0.01	< 0.01	0.05

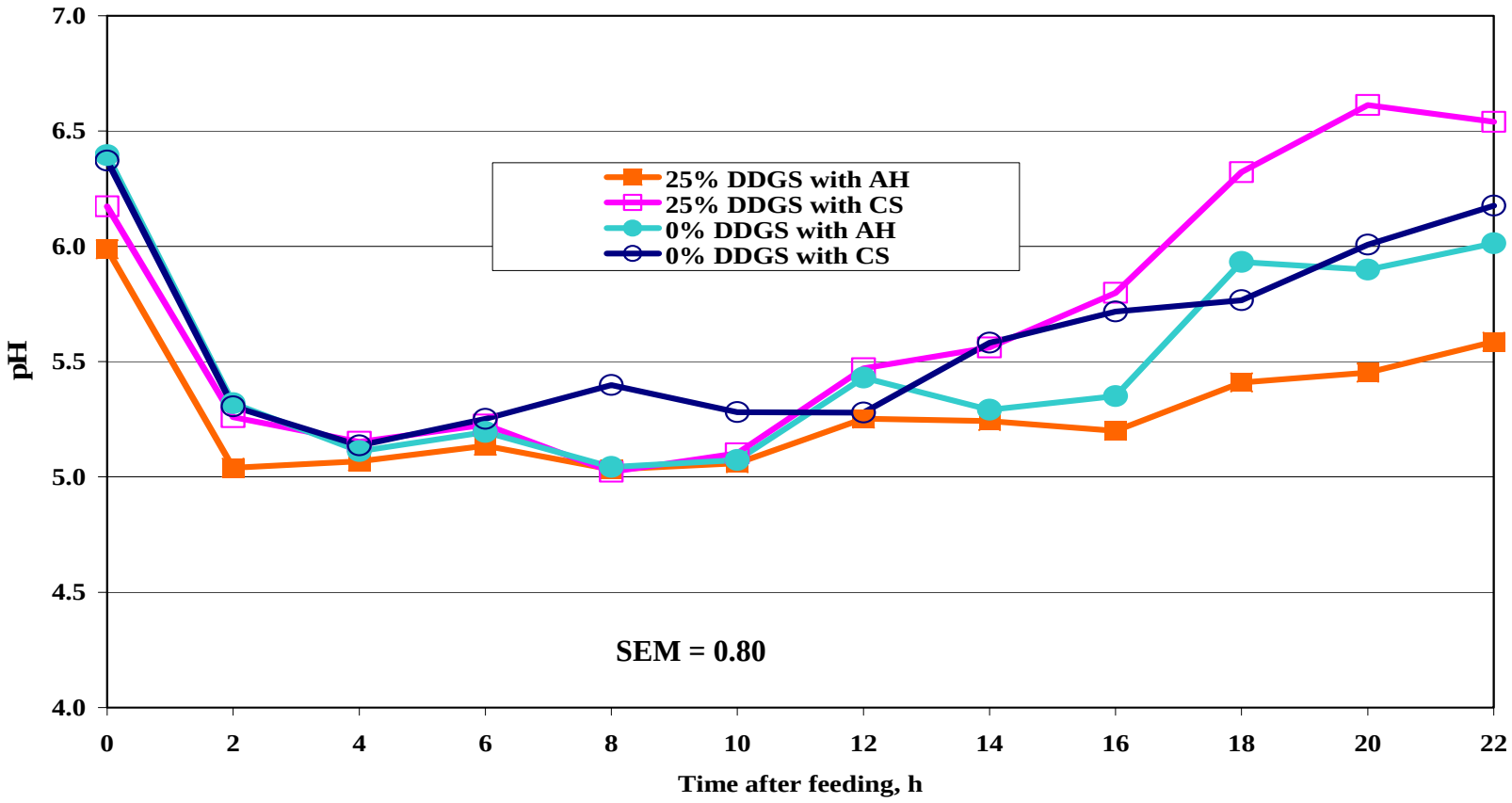
[a] When observations are missing, larger SEM is presented

Table 3-5 Composition of *in vitro* inoculum and reagent concentrations in the ruminal medium[a]

Component	Inoculum concentration (amount/L)	Final medium concentration
Strained rumen fluid	450 mL	300 mL/L
Buffer extract of rumen solids	450 mL	300 mL/L
McDougall's buffer	0	400 mL/L
2-mercaptoethanol*	234 mg	2.0 mM
Maltose solution (100 mg/l)*	50 mL	3.3 mg/mL
Hydrazine solution (60 mM)*	25 mL	1.0 mM
Chloramphenicol solution (1.80 mg/ml)*	25 mL	30.0 µg/mL

[a] Adapted from Broderick, (1987)* Maltose, hydrazine sulfate, and chloramphenicol solutions were prepared in McDougall's buffer. Reagents were added into the inoculum in the following order: 2-mercaptoethanol, maltose, hydrazine sulfate, and chloramphenicol

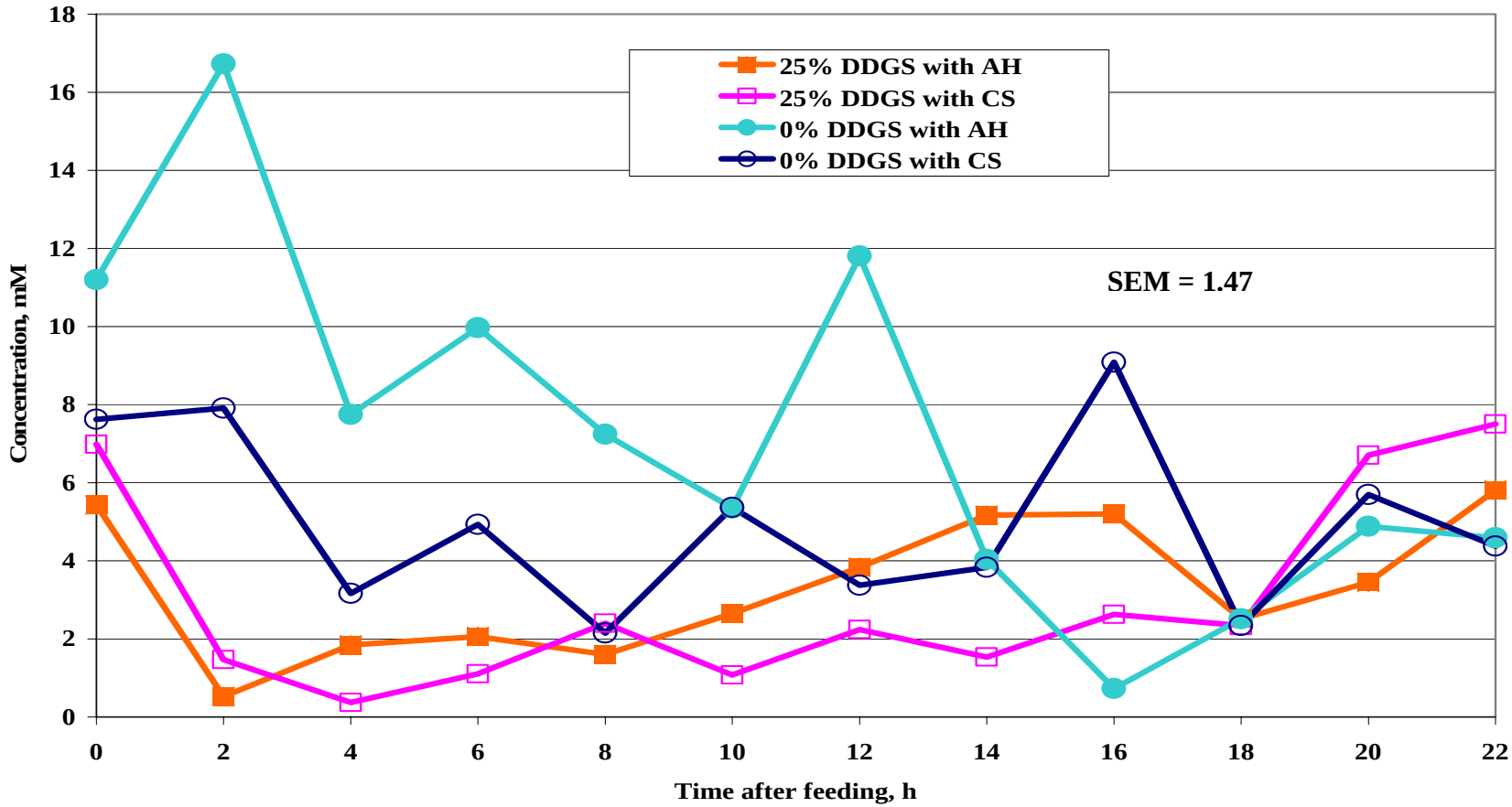
Figure 3-1 Ruminal pH in cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources [a,b]



[a] Interactions between roughage source, DDGS level, and time post feeding ($P < 0.05$)

[b] Interaction between roughage source and DDGS level ($P < 0.05$)

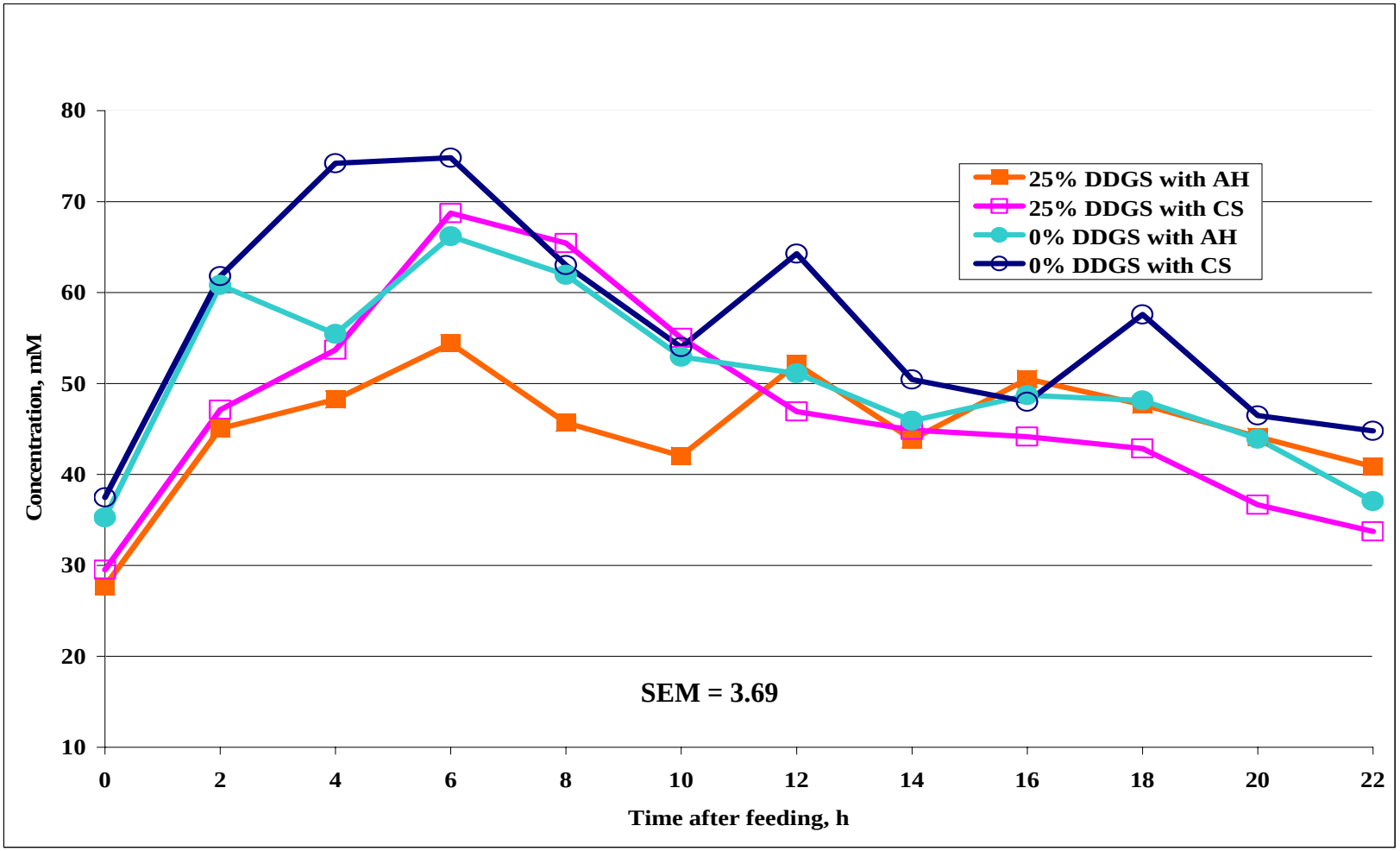
Figure 3-2 Ruminal ammonia concentrations in cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources [a, b]



[a] Interaction between DDGS level and time post feeding ($P < 0.05$)

[b] Interaction between roughage source and DDGS level ($P < 0.05$)

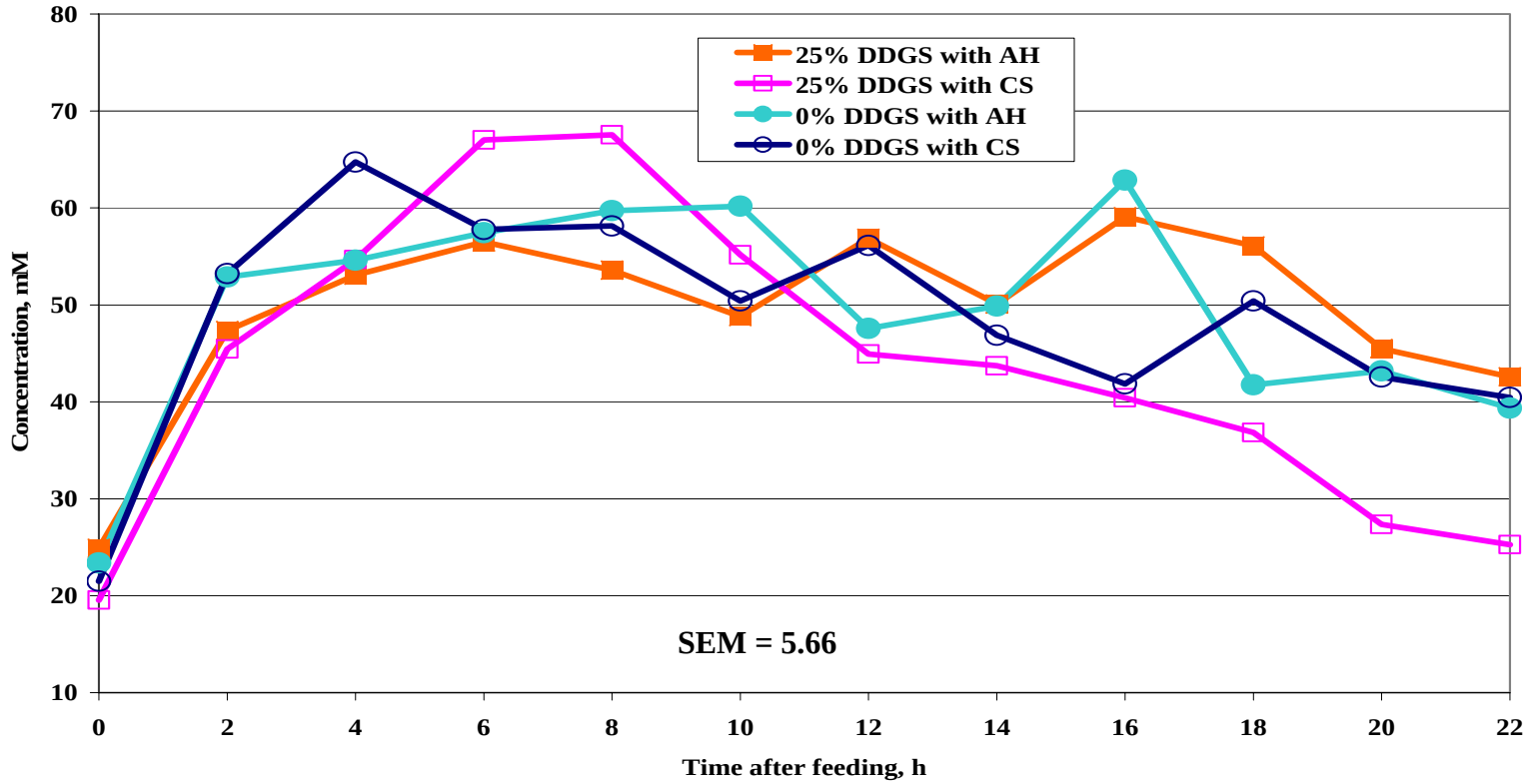
Figure 3-3 Ruminal acetate concentrations in cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources [a, b]



[a] Effect of DDGS level ($P < 0.05$)

[b] Effect of time after feeding ($P < 0.05$)

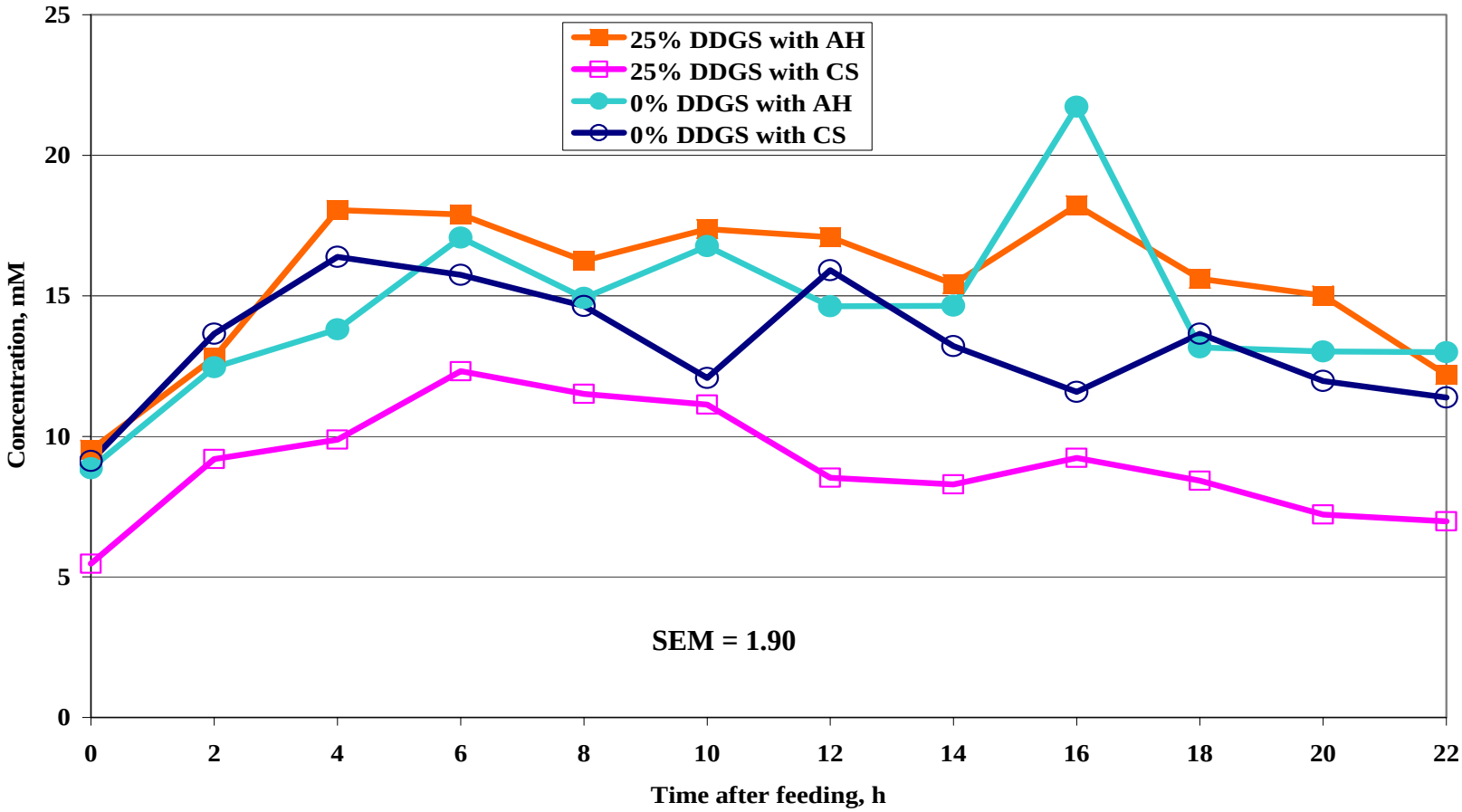
Figure 3-4 Ruminal propionate concentrations in cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources [a, b]



[a] Interaction between roughage source and DDGS level ($P < 0.05$)

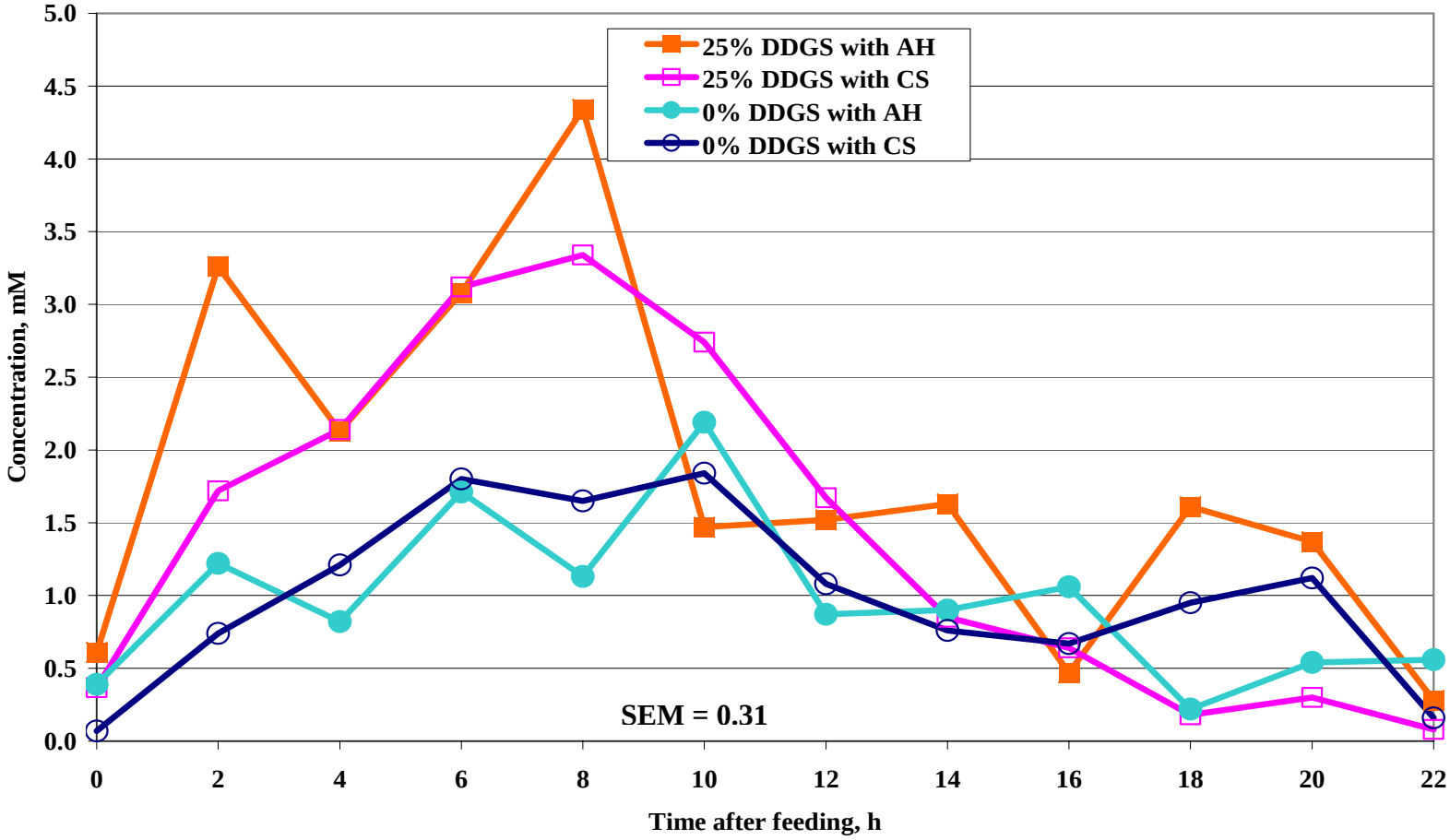
[b] Effect of time after feeding ($P < 0.05$)

Figure 3-5 Ruminal butyrate concentrations in cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources [a]



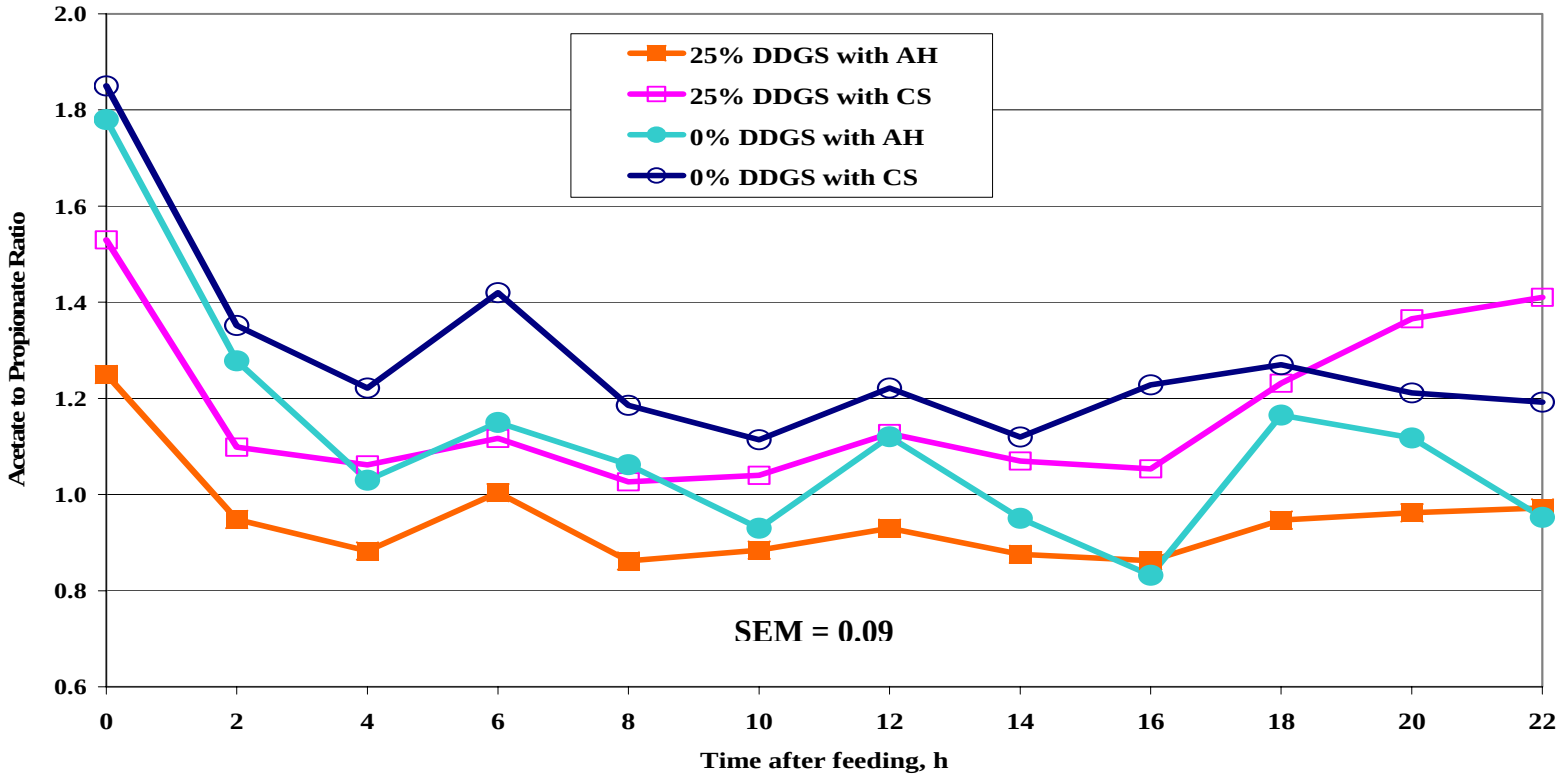
[a] Interaction between roughage source and DDGS level ($P < 0.05$)

Figure 3-6 Ruminal lactate concentrations in cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources [a]



[a] Interaction between DDGS level and time after feeding ($P < 0.05$)

Figure 3-7 Acetate:Propionate ratio in cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources [a, b, c]

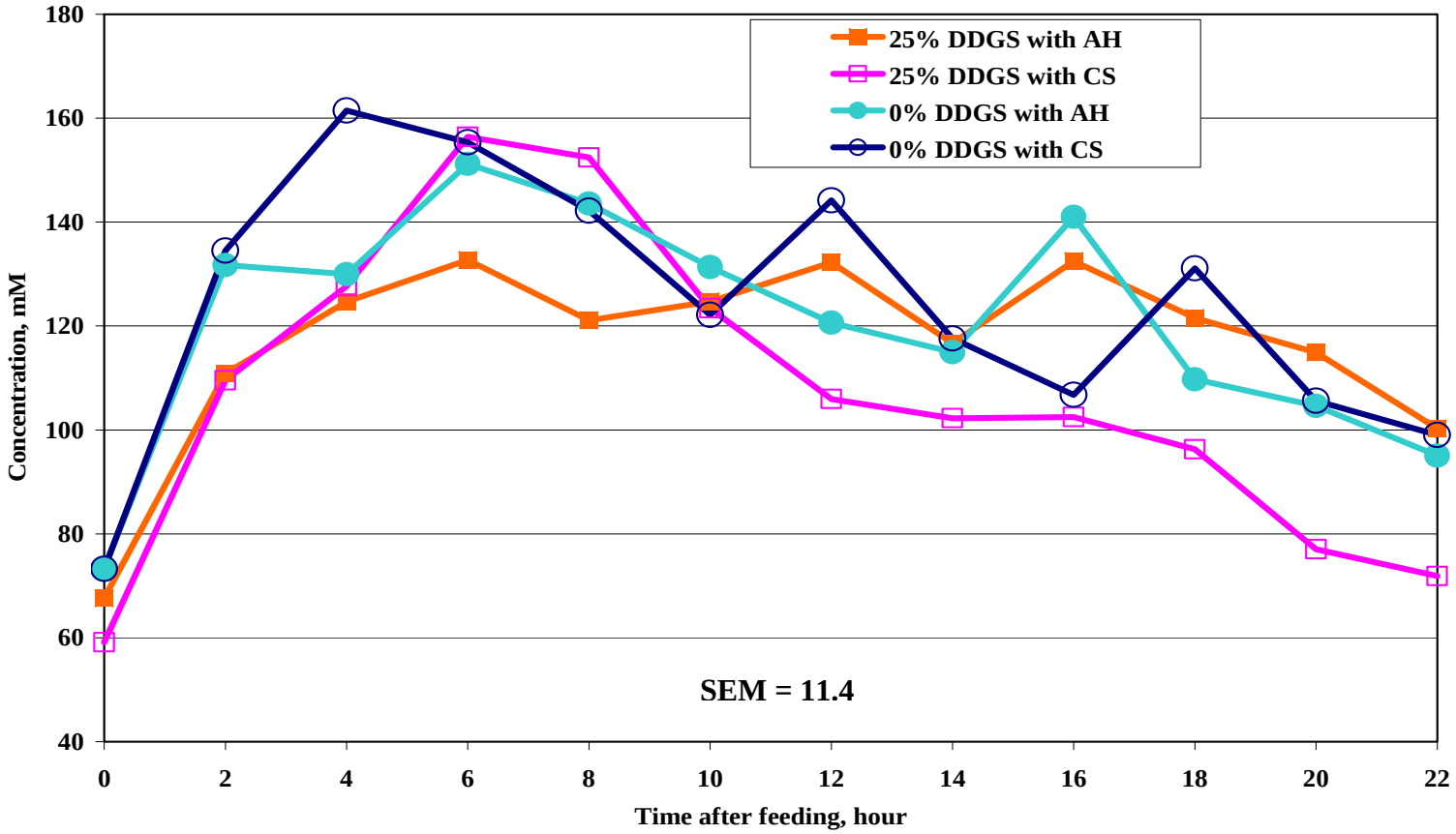


[a] Effect of DDGS level (P< 0.05)

[b] Effect of time after feeding effect (P < 0.05)

[c] Effect of roughage source (P < 0.05)

Figure 3-8 Total Ruminal VFA concentrations in cannulated Holsteins steers fed steam-flaked corn diets with 0 or 25% (DM basis) dried distiller's grains with solubles using alfalfa hay or corn silage as the roughage sources [a, b]



[a] Interaction between roughage source and DDGS level ($P < 0.05$).

[b] Effect of time post feeding ($P < 0.05$).

CHAPTER 4 - Effect of pH on *in vitro* fermentative activity of ruminal contents from cattle adapted to finishing diets containing dried distiller's grains with solubles

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This is contribution No…. -J from the Kansas Agricultural Experiment Station, Manhattan. KS

Abstract

Ruminal pH typically is lower in cattle fed flaked grain diets compared to cattle fed rolled grain diets. We hypothesized that low ruminal pH may restrict digestion of dried distiller's grains with solubles (DDGS), potentially explaining interactions between distiller's grains and grain processing methods. A study was conducted to investigate effects of pH on *in vitro* fermentative activity of ruminal contents from cattle adapted to a finishing diet containing 25% (DM basis) DDGS. The study was a randomized complete block design with a 2 x 3 x 4 factorial treatment arrangement. Factors were buffer type (citric buffer or phosphate buffer), pH level (5, 5.5, or 6) and fermentation time (6, 12, 24, or 48 h), and sampling day served as a block. A 50:50 mixture of DDGS and dry-rolled corn was used as substrate. Fermentations consisting of a 2:1 mixture of McDougall's buffer and ruminal fluid were adjusted to target pH using citric acid or phosphoric acid. Fermentations were duplicated on each of the 3 d (6 observations/treatment, for each buffer). Concentrations of VFA and *in vitro* disappearance of DM (IVDMD) were measured. There was an interaction ($P < 0.01$) between pH and fermentation time with respect to A:P ratio regardless of the buffer type, and concentrations of acetate, propionate, valerate, and total VFA when citric buffer was used. VFA concentrations were higher for pH 5.5 and 6.0 fermentations after 6 and 12 h, but were higher for pH 5.0 fermentations after 24 and 48 h only when citric buffer was used. IVDMD increased with increasing pH (Lin, $P < 0.01$; Quad, $P < 0.01$) and fermentation time. These results may help to explain decreases in cattle performance and diet digestibility when distiller's grains are substituted for steam-flaked grains. Citric buffer should be used only when investigating IVDMD but it is not better suited for VFA analysis compared to phosphate buffer because it serves as a substrate for ruminal microorganisms.

Introduction

Steam-flaking grain was ranked as the most prevalent grain processing method used in feedlot operations, followed by high moisture corn and dry-rolled corn in a survey of 29 consulting nutritionists by Vasconcelos and Galyean (2007). Flaking grain results in a 9 to 18% increase in starch digestion within the rumen compared to ground or cracked corn, and total tract digestion of grain is greater with steam-flaking (99%) compared to dry-rolling (94%) or fine grinding (94%; Theurer, 1986). While comparing dry-rolled corn (DRC) to steam-flaked corn (SFC) at two levels of feed intake, Zinn et al. (1995) observed that ruminal degradation and total tract digestion of OM and starch, as well as NEm and NEg were improved with SFC compared to DRC. Fecal excretion was greater for DRC compared to SFC.

Advantages of steam-flaking grain are less with respect to growth performance and diet digestibility when a portion of grain is replaced with distiller's grains (Lodge et al., 1997; Al-Suwaiegh et al., 2002; May, 2007). Ruminal pH typically is lower in cattle fed flaked grain diets compared to cattle fed rolled grain diets (May 2007). Ruminal pH for cattle fed finishing diets based on steam-flaked corn is observed below pH 6.0 (Corona et al., 2006; Sindt et al., 2006). Depenbusch et al. (2007) observed a 13% decrease in total tract digestibility of DM and OM when 13% DDGS on DM basis was added to SFC-based finishing diets. A decline in ruminal pH below 6.2 reduces activity of fibrolytic organisms (Russell, 1996). Furthermore, ruminal proteolysis declines with pH below 5.5 (Bach et al., 2005). It is plausible that low ruminal pH may restrict digestion of DDGS in flaked grain diets due to its high content in NDF and yeast protein which underwent heat-denaturation. The objective of this study was to examine effects of pH on *in vitro* fermentative activity of ruminal contents from cattle adapted to a finishing diet containing 25% (DM basis).

Materials and Methods

Procedures followed in the present study were approved by the Kansas State University Institutional Animal Care and Use Committee protocol no. 2535.

A study was conducted to investigate effects of pH on *in vitro* fermentative activity of ruminal contents from cattle adapted to a finishing diet containing 25% DDGS (DM basis). The study was a randomized complete block design with a 2 x 3 x 4 factorial treatment arrangement. Factors consisted of buffer type (citric buffer or phosphate buffer), pH level (5.0, 5.5, or 6.0) and fermentation time (6, 12, 24, or 48 h). Sampling day served as a block. There were 2 tubes containing substrate and 2 blank tubes (without substrate) for each of the buffer types, each of the four fermentation times, and each of the three pH levels. The experiment was repeated on three separate days (6 observations/treatment for each buffer type). Because citric acid is metabolizable in the rumen, it is conceivable that citrate degradation may produce some VFAs, hence phosphate buffer was used as a control especially for analysis of VFA profile.

The substrate was a 50:50 mixture of dry-rolled corn (DRC) and DDGS. Samples of DRC and DDGS were analyzed for 105°C DM while another set of both DRC and DDGS samples were dried at 55^{o}C for 24 hours and ground through a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia PA) prior to being blended in a 50:50 ratio. Dry weight of centrifuge tubes was recorded and 0.5 g of the substrate was weighed therein. Fermentations consisting of a 2:1 mixture of McDougall's buffer (El-Shazly and Hungate, 1965) and ruminal fluid were adjusted to the target pH (5.0, 5.5, or 6.0) using citric acid or phosphoric acid. Whole ruminal contents were obtained from a ruminally cannulated steer fed a SFC-based finishing diet with 25% DDGS (DM basis). The diet composition is further described in Table 4.1. Ruminal contents were strained through eight layers of cheesecloth, and pH was immediately recorded prior to bubbling strained ruminal fluid (SRF) with carbon dioxide to purge air. McDougall's

buffer was prepared as described by El-Shazly and Hungate (1965) and adjusted to the desired pH using citric buffer or phosphate buffer as described by Grant and Mertens (1992). The approximate amount used to attain pH 6.0; 5.5; and 5.0 using citric buffer are as follows: For pH 6.0, to 960 mL of the buffer solution described by El-Shazly and Hungate (1965), add 40 mL of 1 M citric acid. For pH 5.5, to 947 mL of the buffer solution add 53 mL of 1 M citric acid, and for pH 5.0, add 67.5 mL of 1 M citric acid to 932.5 mL of the buffer solution. The approximate amount used to attain pH 6.0; 5.5; and 5.0 using phosphate buffer are as follows: For pH 6.0, to 991.4 mL of the buffer solution described by El-Shazly and Hungate (1965), add 8.6 mL of 1 M phosphoric acid. For pH 5.5, to 991.2 mL of the buffer solution add 8.8 mL of 1 M phosphoric acid, and for pH 5.0, add 9.0 mL of 1 M phosphoric acid to 990 mL of the buffer solution. Prior to *in vitro* experiments, buffer solutions were checked to verify pH and to allow for additional bicarbonate or acid addition necessary to attain target pH of the buffer solution. Two 50 mL volumes of each buffer were mixed and incubated at 39°C under carbon dioxide for 4 h, and pH changes were monitored throughout the 4-h fermentation period. Buffer solutions were prepared immediately prior to being mixed in a 2:1 ratio with SRF. The pH values of resulting mixtures were checked and readjusted to the target pH. An aliquot of 30 mL of this mixture was added to centrifuge tubes containing 0.5 g of substrate, bubbled with CO_2, capped with gas-release stoppers, and placed into a in shaking water bath at 39°C. After each time point, tubes were immediately placed in an ice water bath to cease fermentation rapidly while taking final pH. After cooling, tubes were centrifuged at 30,000 x g for 20 min. Supernatant was decanted and an aliquot of 4 mL were mixed with 1 mL of 25% (w/v) metaphosphoric acid for subsequent analyses of VFA. Pellets which remained in the tubes were dried at 100^{o}C overnight, put into

desiccators to cool at room temperature, and weighed to measure *in vitro* dry matter disappearance. The *in vitro* dry matter disappearance (IVDMD) was computed:

$$\text{IVDMD, \%} = \frac{A - [(B - C) - (D - E)]}{A} \times 100$$

Where, A = initial sample weight; B = final weight of sample and tube after drying and desiccation; C = weight of the empty dry tube containing sample; D = weight of blank tube with its content after drying and desiccation; and E = weight of the empty blank dry tube.

The acidified ruminal fluid samples were immediately frozen at -20°C and retained for later analyses. Upon thawing, acidified ruminal fluid was centrifuged at 30,000 × g for 15 min and the supernatant was analyzed for acetate, propionate, isobutyrate, butyrate, isovalerate, valerate, and lactate by gas chromatography (Hewlett-Packard 5890A, Palo Alto, CA; 2 m x 2 mm column; Supelco Carbopack B-DA 80/120 4% CW 20 m column packing, Bellefonte, PA), using He as the carrier gas, a flow rate of 24 mL/min, and a column temperature of 175°C. Total VFA production was computed as the sum of individual VFAs.

In vitro dry matter disappearance and VFA profiles were analyzed using the mixed procedure of SAS version 9.1. (SAS Inst. INC., Cary, NC). Centrifuge tube was the experimental unit, and sampling day was used as the random effect. The model statement included pH level, fermentation time, and pH level x fermentation time interaction. Pre-planned contrasts included pH 5.0 vs the mean of pH 6.0 and 5.5 to verify if there are response differences at pH close to 5, which is usually observed in cattle fed SFC-based diets, and at pH between 5.5 and 6.0 which is normally observed in cattle fed DRC- based diets. Linear and quadratic effects of pH also were tested to characterize the relationship between pH level and digestion characteristics. Means

separations were *F*-test protected ($P \leq 0.05$). Treatment means were determined by using LSMEANS option.

Results and Discussion

The pH changes throughout fermentation were 0.04; 0.04; 0.10; and 0.25 pH unit for 6, 12, 24 and 48 h for citric buffer which confirms the stability of citric buffer solutions used for fermentation. Phosphate buffer was less stable than citric buffer, and pH changes were 0.22; 0.27; 0.32; and 0.39 pH units for 6, 12, 24, and 48 h, respectively. The difference is likely due to the amount of citric acid or phosphoric acid used to adjust Mc Dougall's buffer to pH targets. Compared to the amount of phosphate used, more citric acid was used to attain desired pH targets.

No pH x fermentation time interaction was observed ($P > 0.20$) with respect to IVDMD when citric buffer was used (Figure 4.3) or when phosphate buffer was used (Figure 4.6). For citric buffer, i*n vitro* dry matter disappearance increased with increasing pH (Lin, $P < 0.01$; Quad, $P < 0.01$) and fermentation time ($P < 0.01$), but IVDMD was not affected by pH level when phosphate buffer was used ($P > 0.20$). In addition, IVDMD for pH 5.0 fermentation was lower ($P < 0.01$) than IVDMD for pH 5.5 and 6.0 fermentations combined together at each time point.

Mertens and Loften (1980) investigated the effect of starch on kinetics of forage fiber digestion *in vitro.* Lag time of fiber digestion increased with increasing addition of starch and the potential extent of digestion was decreased with starch addition. The linear increase of IVDMD as pH increases may indicate that fibrolytic activity declines at pH below 6.0, as discussed previously, which might have affected digestion of DDGS present in substrate. More

importantly, low IVDMD may indicate that protein degradation is inhibited at low pH which may affect subsequent digestion of ruminal digesta; hence low IVDMD observed at low pH.

Concentrations of acetate, propionate, butyrate, and lactate when citric buffer was used are summarized in Table 4.2 and in Table 4.4 when phosphate buffer was used. There was interaction between pH level and fermentation time ($P < 0.01$) with respect to *in vitro* concentrations of acetate and propionate when citric buffer was used, but acetate and propionate concentrations were not affected by pH levels ($P > 0.10$) when phosphate buffer was used. For the first 12 h, acetate concentration increased for pH 6.0, but it dropped after 24 h. Propionate concentration continued to increase up to 48 h of fermentation for pH 5.0 and 5.5 fermentations, but it dropped between 24 and 48 h for pH 6.0. There was a pH level x fermentation time interaction ($P = 0.05$), with respect to A:P ratio when citric buffer was used. Acetate:propionate ratio was low ($P < 0.01$) for pH 5.0 compared to the average A:P ratio of pH 5.5 and pH 6.0. The A:P ratio increased linearly ($P < 0.01$) as pH increased the first 6 h, and quadratically ($P < 0.01$) until 12 h while it decreased quadratically between 24 and 48 h ($P < 0.01$, Figure 4.1). When phosphate buffer was used, A:P ratio increased linearly as pH increased ($P = 0.05$; Figure 4.4). Butyrate concentration increased with increasing pH (Linear, $P = 0.01$) when citric buffer was used, and with fermentation time ($P < 0.01$) regardless of buffer type. Butyrate concentration for pH 5.0 was lower ($P = 0.03$) compared to the average concentration for pH 5.5 and pH 6.0 in case of citric buffer.

Bhatti and Firkins (1995) demonstrated that the digestion of NDF in distiller's grains is initially slow. These authors suggested that the slow initiation could be an indication of the low water holding capacity (0.062g/g of insoluble DM) of NDF in distiller's grains, since fiber must be hydrated before digestion by bacteria, which may explain the lag phase we observed in VFA

production. However, because DDGS content was similar, pH might have become a limiting factor for digestion of the substrate. Not only the activity of fibrolytic bacteria is inhibited at low pH (Huang et al., 1988), but also proteolysis declines at pH lower than 5.5 (Bach et al., 2005); hence low acetate, propionate, and butyrate productions observed at low pH. Because there was no absorption of VFA *in vitro*, the decline of acetate concentration may indicate that it was metabolized to some extent. A small amount of acetate can be metabolized in succinate or oxaloacetate in the rumen (Bergman et al., 1965). Additionally, acetate: propionate ratio in the rumen has an inverse relationship with methanogenesis (Lana et al., 1998; Russell, 1998). This relationship is indicated by a lower acetate:propionate ratio and pH (Moss et al., 1995; Lana et al., 1998).

No interactions between pH level and fermentation time were observed ($P > 0.20$) with respect to the concentrations of isobutyrate, isovalerate, and valerate, regardless of buffer types (Table 4.3 and Table 4.5). Both isobutyrate and isovalerate concentrations increased linearly ($P < 0.01$) with increasing pH and fermentation time ($P < 0.01$) when citric buffer was used, but they were not affected by pH level ($P > 0.10$) when phosphate buffer was used. The concentrations of isobutyrate and isovalerate were lower ($P < 0.01$) for pH 5.0 compared to the average concentrations for pH 5.5 and 6.0 at each time point in case of citric buffer. Valerate concentration increased with increasing fermentation time ($P < 0.01$), but was not affected by levels of pH ($P > 0.20$) in both buffer types. Branched-chain VFAs are the end-products of branched-chain amino acids degradation. An increase in concentrations of these VFAs as pH increases may indicate that protein degradation was inhibited at low pH (Cardozo et al., 2000; 2002). These researchers conducted 2 dual flow continuous culture fermentation studies

comparing high forage vs. high concentrate rations at pH ranging from 4.9 to 7.0 and demonstrated that protein degradation was reduced as pH decreased with both types of rations.

Total VFA concentrations when citric buffer were used are shown in Figure 4.2 and in Figure 4.5 when phosphate buffer was used. There was interaction between pH level and fermentation time ($P < 0.01$) for total VFA concentrations with use of citric buffer, but were not affected by pH level when phosphate buffer was used. For citric buffer, concentrations were higher for pH 5.5 and 6.0 fermentations after 6 and 12 h, but were higher for pH 5.0 after 24 and 48 h. The average total VFA concentrations were 49, 91, and 93 mM after 6 h for pH 5.0; 5.5; and 6.0 respectively; 103, 115, and 123 mM after 12 h for pH 5.0; 5.5; and 6.0 respectively; 167, 154, and 152 mM after 24 h for pH 5.0; 5.5; and 6.0 respectively; and 170, 158, and 136 mM after 48 h for pH 5.0; 5.5; and 6.0, respectively, when citric buffer was used. When phosphate buffer was used, the average total VFA concentrations were as follows: 53, 71, and 75 mM after 6 h for pH 5.0; 5.5; and 6.0 respectively; 84, 85, and 88 mM after 12 h for pH 5.0; 5.5; and 6.0 respectively; 100, 96, and 91 mM after 24 h for pH 5.0; 5.5; and 6.0 respectively; and 107, 109, and 98 mM after 48 h for pH 5.0; 5.5; and 6.0, respectively.

As pH was lowered, more citrate was added, and citrate being an element of TCA cycle, it presumably served as fermentation substrate for the microbes, and led to greater VFA production. This could readily explain why at 48 h, for pH 5.0, VFA concentrations increase as pH decreases, even though IVDMD is going the opposite pattern. Furthermore, the amount of substrate fermented in phosphate buffer was similar to the amount of substrate fermented in citric buffer, using ruminal fluid from the same steer. Thus the big difference in VFA concentration observed between the two buffers types could indicate that citrate was metabolized by ruminal

bacteria. Although pH effect was not significant when phosphate buffer was used, VFA concentrations numerically increased as pH increased.

Ha et al. (1983) evaluated effects of *in vitro* ground wheat fermentation at pH 4, 5, 6, and 7 for 1, 3, 5, and 7 hours on ruminal lactate and VFA production in sheep. Lowering the incubation pH to below 6 reduced total VFA productions at all time points and increased the acetate to propionate ratio which is consistent with our observations at the earlier time points. Greater acetate to propionate ratios occurred at pH 5 than at pH 7 during the first 3 h of incubation, and ratios were greater at pH 4 than at pH 7 for the entire 7 h. Ha et al. (1983) also suggested that higher A:P ratio was due to a disproportionately greater reduction in propionate production at the lower pH values. Butyrate was not affected by variations in pH. Slyter et al. (1966) reported that lower ruminal pH depresses total VFA production and the ratio of acetate to propionate. In vitro studies summarized by Russell and Wilson (1996) suggest a rapid decline in activity of fibrolytic organisms when pH fell below 6.2. In addition, the optimal pH range for cellulases of ruminal bacteria is rarely below pH 6.0 (Huang et al., 1988; McGavin; Forsberg, 1988; McGavin et al., 1989).

Lactate concentration in our study was very low throughout the 48-h fermentation period with use of citric buffer (Table 4.2) and phosphate buffer (Table 4.4), and was not affect by pH ($P > 0.10$) regardless of buffer type. Unlike our results, in a study by Ha et al. (1983), reducing incubation pH from 6 to 5 resulted in accumulation of lactate, but further reduction to pH 4 did not result in an additional increase in lactate production. According to Russell et al. (1979), the major lactate-producing bacterium *Streptococcus bovis* stops growing at pH below approximately 5.1 which may explain the low lactate concentrations observed at pH 5.0 in our study. Lactate can also be metabolized to propionate or butyrate especially at low pH. If any

lactate was produced, it is possible that the lactate was metabolized to propionate, which is supported by the constant increase of propionate observed at pH 5.0 and 5.5 over time.

Conclusions

Higher pH led to greater dry matter disappearance *in vitro*. These results may help to explain decreases in cattle performance and diet digestibility when distiller's grains are combined with cereals that result in low ruminal pH, as is the case with flaked grains. Feeding strategies aimed at increasing ruminal pH may constitute a logical approach to improving digestion of DDGS in flaked-grain finishing diets. Citric buffer is good for use only when investigating IVDMD but it is not better suited for VFA analysis because it serves as a substrate for ruminal microorganisms.

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Table 4-1 Composition of the diet fed to the cannulated steer donor of the ruminal fluid

Ingredients	% Dry matter
Steam flaked corn	58.3
Corn dried distiller's grains	25.1
Alfalfa hay	5.8
Corn steep liquor	6.3
Urea	0.1
Limestone	1.7
Supplement[1]	2.7
Analyzed composition, %	
Dry matter	79.2
Crude protein	16.0
Ether extract	5.4
NDF	15.6
Calcium	0.7
Phosphorus	0.5
Potassium	0.7

[1] Formulated to provide 300 mg/day monensin, 90 mg/day tylosin, 2,200 IU/kg vitamin A, 0.3 % salt, 22 IU/kg vitamin E, 60 mg/kg Mn, 60 mg/kg Zn, 0.63 mg/kg I, 0.25 mg/kg Se, and 0.1 mg/kg Co.

Table 4-2 Effect of pH on major VFA and lactate concentrations from *in vitro* fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using citric buffer

	Fermentation time, hours					P- values/ Contrasts					
Item	6	12	24	48	SEM	pH effect	Fermentation time effect	pH x time interaction	pH 5.0 vs average of pH 5.5 and 6.0	pH Linear	pH Quadratic
Acetate, mM					6.74	0.46	< 0.01	< 0.01	0.34	0.67	0.24
pH 5	26.9	64.0	103.6	100.4							
pH 5.5	56.5	74.5	93.4	91.9							
pH 6	58.3	75.5	89.6	78.8							
Propionate, mM					3.80	0.67	< 0.01	< 0.01	0.38	0.44	0.65
pH 5	11.1	18.8	32.0	33.5							
pH 5.5	16.2	19.0	27.6	28.6							
pH 6	16.2	22.2	27.7	24.9							
Butyrate, mM					1.80	0.06	< 0.01	0.10	0.03	0.01	0.08
pH 5	7.5	14.0	21.0	23.0							
pH 5.5	12.3	15.2	22.3	24.5							
pH 6	12.2	17.0	22.4	20.1							
Lactate, mM					0.29	0.52	0.96	0.54	0.32	0.26	0.97
pH 5	0.28	1.12	0.21	0							
pH 5.5	0.63	0.40	0	0.1							
pH 6	0.18	0.30	0.11	0.1							

Table 4-3 Effect of pH on minor VFA concentrations from *in vitro* fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using citric buffer

	Fermentation time, hours					P-values/ Contrasts					
VFA, mM	6	12	24	48	SEM	pH effect	Fermentation time effect	pH x time interaction	pH 5.0 vs average of pH 5.5 and 6.0	pH Linear	pH Quadratic
Isobutyrate					0.13	< 0.01	< 0.01	0.37	< 0.01	< 0.01	0.48
pH 5	0.41	0.70	1.10	1.62							
pH 5.5	0.67	0.83	1.41	1.78							
pH 6	0.69	1.06	1.70	1.72							
Isovalerate					0.63	<0.01	<0.01	0.43	< 0.01	< 0.01	0.86
pH 5	1.67	3.07	4.33	5.62							
pH 5.5	2.77	3.15	4.82	6.04							
pH 6	2.78	3.92	5.95	5.90							
Valerate					0.89	0.67	< 0.01	0.06	0.39	0.52	0.54
pH 5	1.39	2.70	4.51	6.07							
pH 5.5	2.08	2.51	4.35	4.87							
pH 6	2.01	3.05	4.54	4.42							

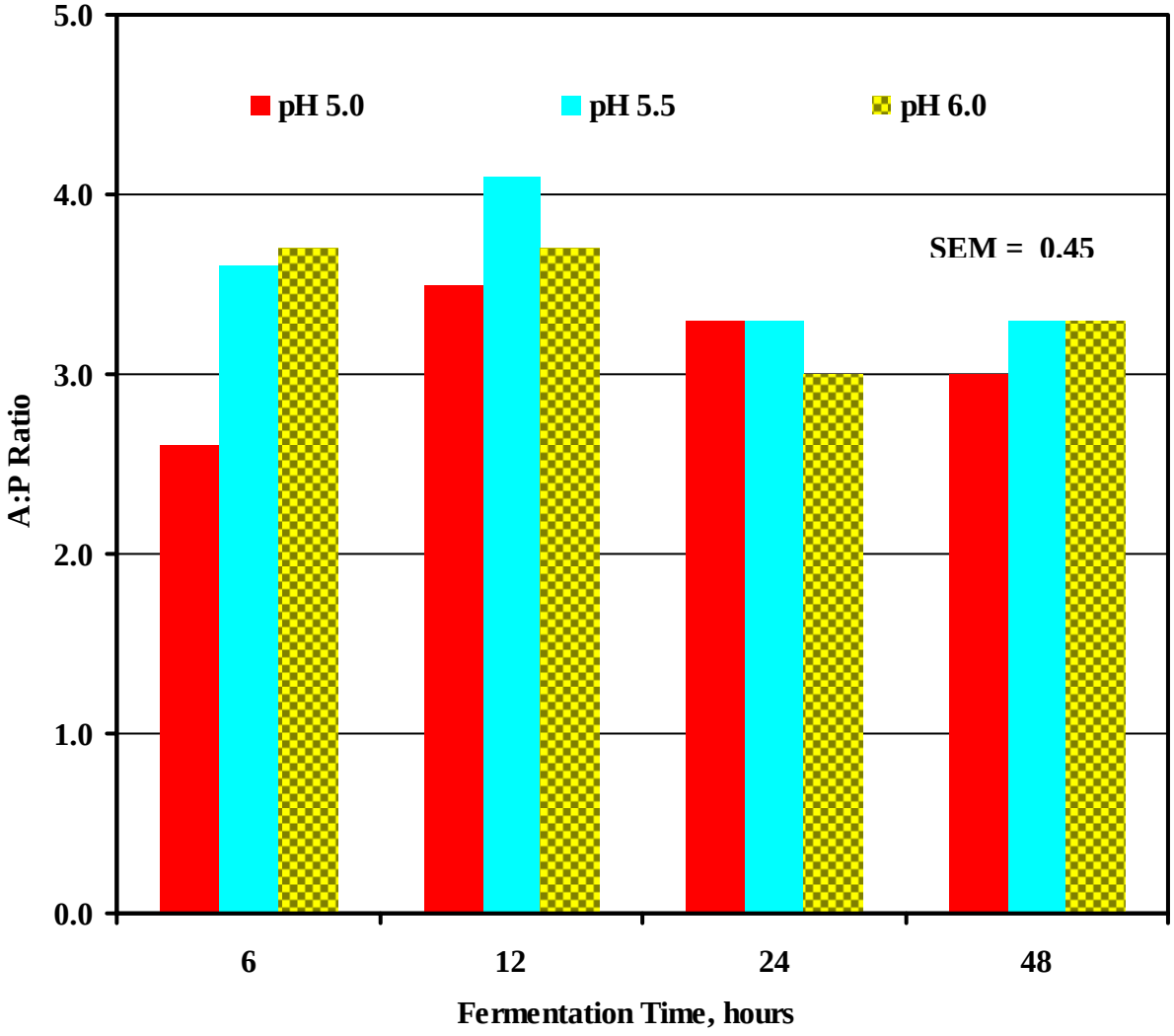
Table 4-4 Effect of pH on major VFA and lactate concentrations from *in vitro* fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using phosphate buffer

	Fermentation time, hours					P- values/ Contrasts					
Item	6	12	24	48	SEM	pH effect	Fermentation time effect	pH x time inter action	pH 5.0 vs average of pH 5.5 and 6.0	pH Linear	pH Quadratic
Acetate, mM					3.83	0.76	< 0.01	0.61	0.47	0.51	0.69
pH 5	26.0	39.9	45.9	47.3							
pH 5.5	34.3	40.4	43.7	48.0							
pH 6	36.8	42.1	43.3	44.6							
Propionate, mM					3.45	0.75	< 0.01	0.39	0.91	0.83	0.51
pH 5	13.5	22.3	27.2	17.1							
pH 5.5	18.5	22.1	25.7	22.5							
pH 6	19.4	23.1	22.6	25.2							
Butyrate, mM					1.32	0.68	< 0.01	0.54	0.51	0.74	0.40
pH 5	8.8	14.3	17.1	18.64							
pH 5.5	11.9	14.5	16.7	19.2							
pH 6	12.5	14.9	15.8	17.1							
Lactate, mM					0.52	0.75	0.03	0.69	0.73	0.98	0.44
pH 5	0.62	1.01	0.04	0.04							
pH 5.5	1.91	0.41	0.11	0.03							
pH 6	1.02	0.44	0.07	0.03							

Table 4-5 Effect of pH on minor VFA concentrations from *in vitro* fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using phosphate buffer

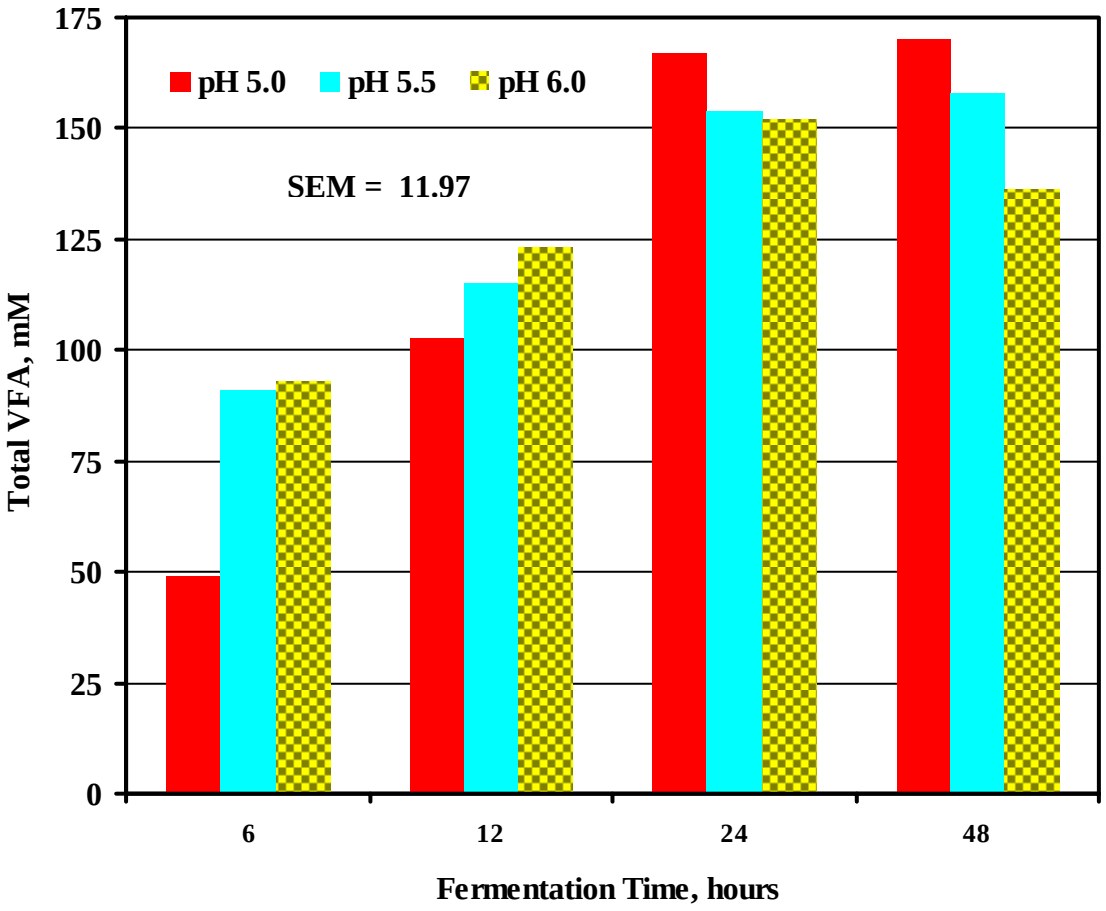
	Fermentation time, hours					P-values/ Contrasts					
VFA, mM	6	12	24	48	SEM	pH effect	Fermentation time effect	pH x time interaction	pH 5.0 vs average of pH 5.5 and 6.0	pH Linear	pH Quadratic
Isobutyrate					0.10	0.89	< 0.01	0.50	0.64	0.81	0.66
pH 5	0.57	0.87	1.17	1.71							
pH 5.5	0.71	0.87	1.10	1.77							
pH 6	0.72	0.95	1.26	1.53							
Isovalerate					0.70	0.76	< 0.01	0.47	0.51	0.64	0.52
pH 5	2.25	3.52	4.50	5.62							
pH 5.5	3.07	3.51	4.29	5.82							
pH 6	3.20	3.71	4.37	5.11							
Valerate					0.70	0.26	< 0.01	0.29	0.77	0.66	0.14
pH 5	2.03	3.02	4.15	5.49							
pH 5.5	2.34	3.17	4.23	6.03							
pH 6	2.54	3.31	3.64	4.71							

Figure 4-1 Effect of pH on A:P ratio from *in vitro* fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using citric buffer



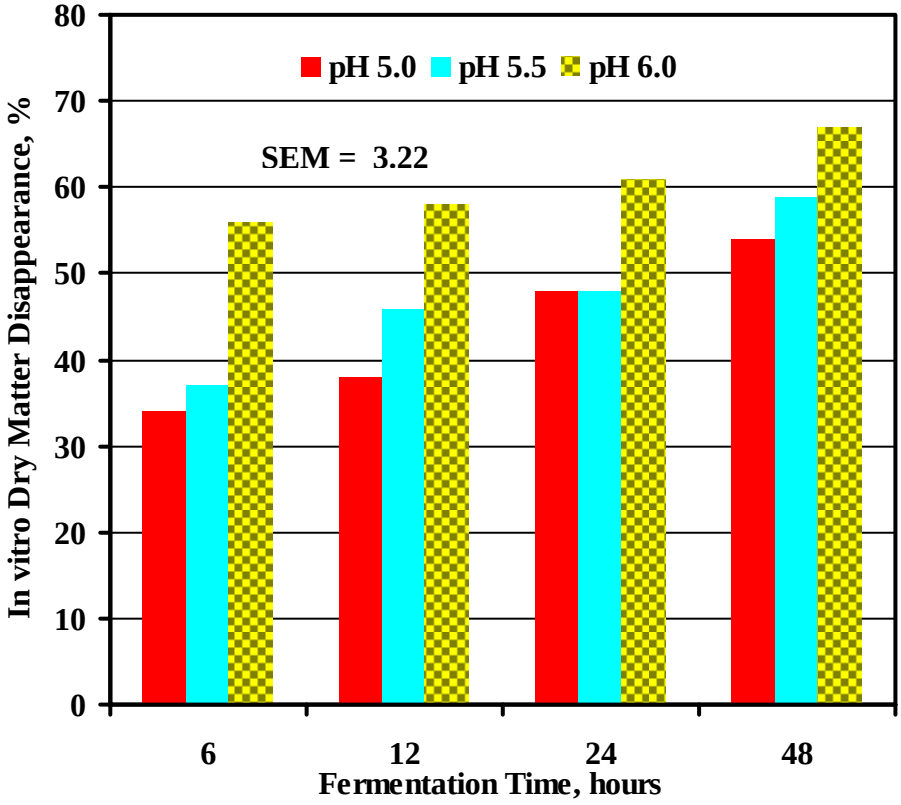
pH effect: Linear, P < 0.01; Quadratic, P < 0.01

Figure 4-2 Effect of pH on total VFA concentrations from *in vitro* fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using citric buffer



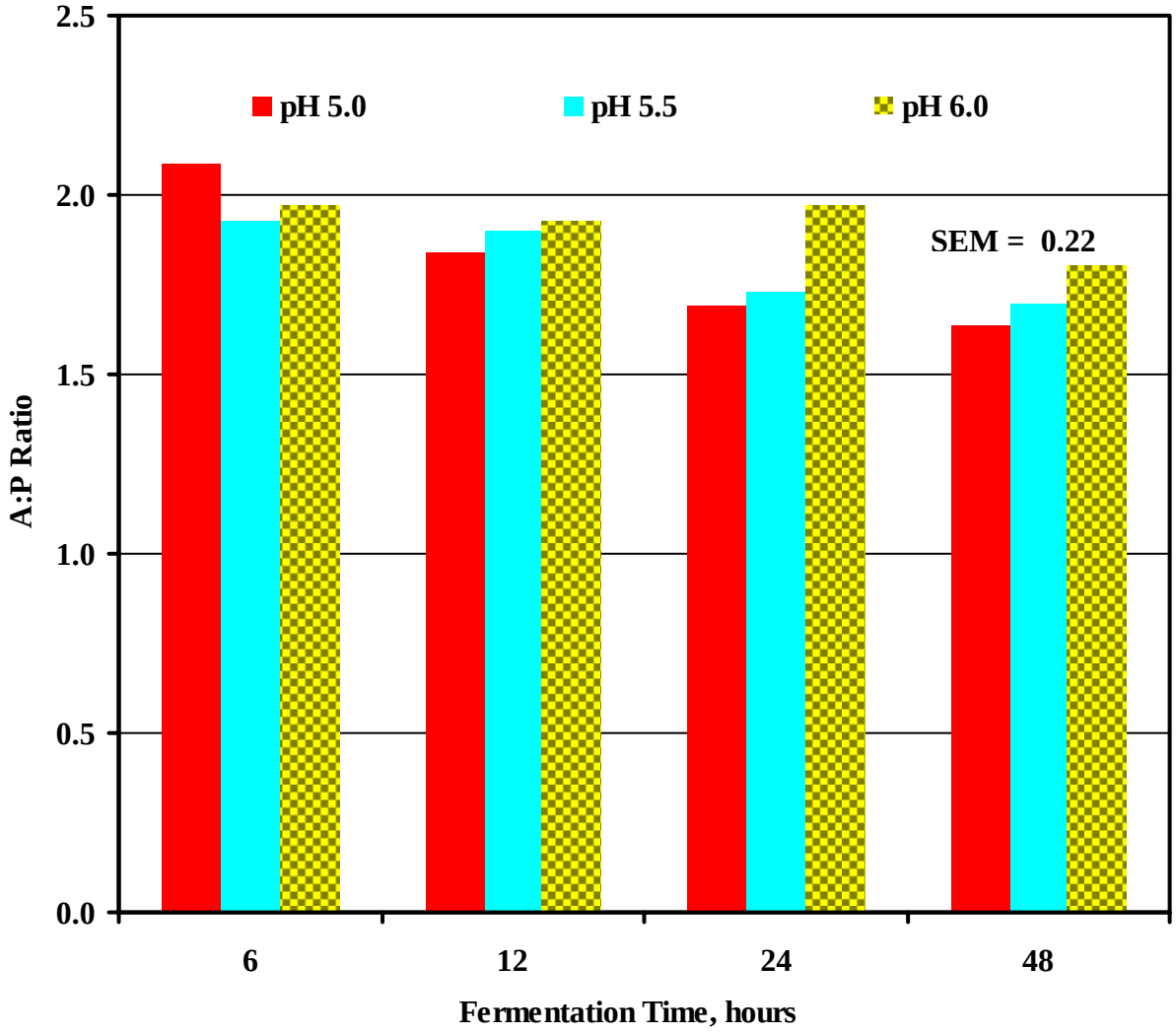
Interaction between pH level and fermentation time, P < 0.01

Figure 4-3 Effect of pH on *in vitro* dry matter disappearance due to fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using citric buffer



pH effect: Linear, P < 0.05; Quadratic, P < 0.01

Figure 4-4 Effect of pH on A:P ratio from *in vitro* fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using phosphate buffer



pH effect: Linear, P = 0.05

Figure 4-5 Effect of pH on total VFA concentrations from *in vitro* fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using phosphate buffer

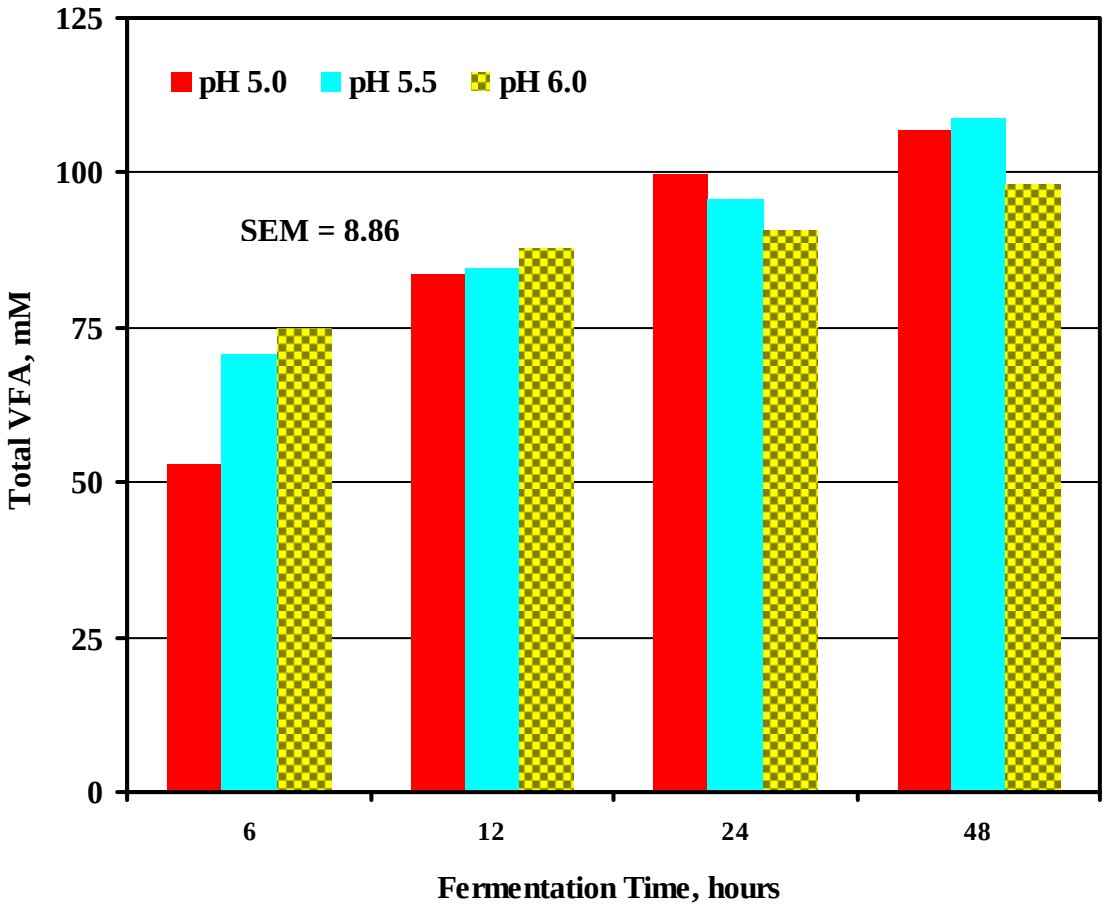


Figure 4-6 Effect of pH on *in vitro* dry matter disappearance due to fermentation by ruminal contents from a steer adapted to a finishing diet containing 25% (DM basis) dried distiller's grains with solubles using phosphate buffer