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Effect of Limit-Fed Co-Product Feedstuffs on Production, Digestion, Fermentation and Rumen Function in Beef Cattle

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by

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ABSTRACT

In terms of energy density, the cost of shipping hay is often not justified in yr where adverse conditions limit available forage. Our objective was to determine if co-product feedstuffs could be used to meet the energy demands for cows in late pregnancy. Eighty-six crossbred cows (527 ± 0.8 kg BW) in late gestation were stratified by BW, BCS and age and allocated randomly to 1 of 6 groups held on 2-ha dormant bermudagrass pastures for 68 d. Three groups were offered bermudagrass hay ad libitum (HAY) and three groups were offered 6.4 kg of soybean hulls (LSH) daily and allowed access to mixed-grass hay for 1 h daily. Changes in BW, BCS, serum non-esterified fatty acids, and birth weights were minimal between treatments ($P \geq 0.12$). In a companion study, 8 ruminally-fistulated cows (671 ± 32.0 kg BW) were stratified by BW and allocated randomly to1 of 4 treatments in a 2-period study: LSH, limit-fed distillers dried grains with solubles (DDGS; LDG), a limit-fed mixture of SH and DDGS (MIX), or ad libitum mixed-grass hay (HAY). Total feces were collected for 5 d following a 28-d adaptation to diet and facilities in each period. Rumen fluid was sampled immediately prior to feeding and 2, 4, 6, 8, 10 and 12 hr post-feeding for ruminal fermentation assessment. Digestibility of DM, OM, aNDF and ADF was greater ($P < 0.05$) from limit-feeding than from those consuming hay. Individual VFA concentrations differed ($P < 0.05$) early in the day, but no difference existed beyond 8 h. In situ forage DM disappearance was reduced ($P < 0.05$) from LSH and LDG in comparison to HAY while diets were being fed. However, cows achieved steady-state forage disappearance within one week following removal from the diets. Based on this information, co-product feedstuffs may be used in lieu of hay to meet the energy requirements of cows during late pregnancy without adverse effects.

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LIST OF ABBREVIATIONS

All abbreviations in this thesis, unless defined within the text, are in accordance with the requirements and format of *Animal Feed Science and Technology*. The following abbreviations represent a compilation of both those approved by the journal and those that are used specifically for this document:

ADF	acid detergent fiber, expressed inclusive of residual ash
ADG	average daily gain
ADIA	acid detergent insoluble ash
aNDF	neutral detergent fiber assayed with heat stable amylase, expressed inclusive of residual ash
B	boron
BCS	body condition score
BW	body weight
Ca	calcium
CH_4	methane
Co	cobalt
CO_2	carbon dioxide
Cu	copper
d	day(s)
DDGS	distillers' dried grains with solubles
DM	dry matter
DMI	dry matter intake
EE	ether extract (crude fat)
Fe	iron
g	gram(s)
g	gravity
h	hour(s)
ha	hectare(s)
I	iodine
K	potassium
kg	kilogram(s)
Lignin (sa)	lignin determined by solubilization of cellulose with sulphuric acid
M	molar (concentration)
ME	metabolizable energy

mM	millimolar
Mg	magnesium
mg	milligram(s)
mL	milliliter(s)
mmol	millimole
Mn	manganese
mol	mole
N	nitrogen
N	normal(ity)
n	number (sample size)
N_2O	nitrous oxide
Na	sodium
NH_3	ammonia
OM	organic matter
P	phosphorus
P	probability
S	sulfur
Se	selenium
SH	soybean hulls
µg	microgram(s)
µL	microliter(s)
WE	water-extractable
wt	weight(s)
yr	year(s)
Zn	zinc

CHAPTER 1: INTRODUCTION

1. Review of Literature

1.1 Limit-feeding

Limit-feeding is a strategy for livestock production that has been in existence, with significant variation, for some time. Galyean (1999) describes two primary categories of limit-feeding: restricted feeding and programmed feeding. In his review, he points out that restricted feeding manages intake with regard to potential or observed ad libitum intake, while programmed intake uses published energy equations (NRC, 2000) to allot feed offering based on maintenance or a prescribed level of production. He goes on to say that restricted feeding finds its niche in the finishing period with feedlot cattle, while programmed feeding is more commonly used in growing programs such as stockering and backgrounding (Galyean, 1999).

Citing a presentation at the Minnesota Nutrition Conference (Owens et al., 1995), Galyean (1999) presents seven potential reasons for employing a limit-feeding strategy. In terms of feedlot programs, avoiding overconsumption, simplifying bunk management, decreasing manure output, identifying potentially sick animals, transitioning between diets and improving feed efficiency would be primary goals. In other situations outside of the feedlot environment, another goal of limit-feeding would be to decrease the use of roughage or pasture.

1.1.1 Efficiency

Many authors have observed discrepancies dealing with efficiency when evaluating limit-feeding programs. When cattle were fed to a specific rate of gain in a feedlot system, ADG was 10 to 35% greater compared with those that were predicted using NRC (1984) net energy equations (Knoblich et al., 1997). Cows that were limit-fed to meet their energy requirements lost wt and BCS (though less than their counterparts fed ad libitum) in some studies (Loerch,

1996), and gained wt and BCS in other studies (Gunter et al., 2000; Schoonmaker et al., 2003). When ewes were limit-fed a high-grain diet during growth, gestation or breeding, ewes lost less wt (Susin et al., 1995a) and less body condition (Susin et al., 1995a; Susin et al., 1995b) compared with their counterparts offered forage for ad libitum consumption.

One reason presented for the observed increased efficiency is that limit-feeding may alter animal behavior or bodily energy expenditures (Hicks et al., 1990). Less energy is expended by the internal organs when the diet is higher in energy or when there is less fill in the gastrointestinal tract (Fluharty and McClure, 1997). Gastrointestinal fill has been used to explain wt loss in cows limit-fed corn without an accompanying loss of condition (Driedger and Loerch, 1999). The same was hypothesized for cows limit-fed corn or corn gluten feed with rice hulls or cottonseed hulls as roughage sources on pasture (Gunter et al., 2000), or heifers offered soybean hulls or corn for limited consumption (Löest et al., 2001), all of which lost wt without the concomitant loss in BCS. While differences in fill have been used to explain fluctuations in wt, this hypothesis does not explain the biology of the matter. Several researchers have examined the wt of visceral organs, believing that the metabolic rate of these organs would decrease with decreasing gut fill (Hicks et al., 1990; Murphy and Loerch, 1994). However, liver and heart wt were not different (Murphy and Loerch, 1994) or tended to increase (Hicks et al., 1990) with limit-feeding.

Animal behavior may have an effect on the overall efficiency of limit-fed cattle. Limit-feeding has been observed to reduce the time spent eating, as well as increase nonrecumbent time by up to 2.1 h/d in heifers (Hoffman et al., 2007). Hicks et al. (1990) however, noted both aggressive and timid behavior patterns between ad libitum and limit-fed pens and thus did not attribute differences in wt change to behavioral alterations. Although receiving the same energy

offering as those fed forage ad libitum, cows receiving limit-fed corn exhibited symptoms of hunger or boredom, such as chewing of tree bark (Loerch, 1996).

1.1.2 Diet digestibility

Differences in diet digestibility in limit-feeding programs have been attributed to the higher energy content of diets fed for limited intake (Galyean et al., 1979). Digestibility of DM was improved by 15% when corn was limit-fed to cows compared with cows offered a high-forage diet (Driedger and Loerch, 1999). Furthermore, DM digestibility from cows was lower from limit-fed DDGS diets compared with limit-fed corn diets (Felix et al., 2011). However, others did not observe a differences in digestibility between limit-fed corn, whole shelled corn (Loerch, 1990; Murphy et al., 1994; Susin et al., 1995b) or wet corn gluten feed (Wertz et al., 2001). In contrast to these data, no differences were observed for limit-fed dry corn gluten feed (Wertz et al., 2001) or from soybean hulls that were limit-fed compared with corn (Löest et al., 2001). With diets fed at levels between 1 and 2 times maintenance, DM and OM digestibility was greatest in the total tract and in the rumen from cows limit-fed at maintenance (Galyean et al., 1979).

Digestibility of NDF was greater from limit-fed DDGS diets compared with corn diets (Felix et al., 2011). Digestibility of ADF improved linearly with whole shelled corn-based diets as the degree of dietary restriction increased (Murphy et al., 1994). This improvement was attributed to either increased ruminal fermentation due to reduced passage rate (Miller and Muntifering, 1985) or enhanced hindgut fermentation (Lewis and Dehority, 1985).

Nitrogen balance data have been inconclusive from limit-feeding research. Nitrogen retention increased linearly in lambs limit-fed whole shelled corn as the degree of dietary restriction increased (Murphy et al., 1994). Likewise, Galyean et al. (1979) reported increased

apparent absorption of N with decreasing intake. However, when gravid Holstein heifers were limit-fed, there were no differences in N intake, absorption or retention compared with heifers consuming diets for ad libitum access, thus limit-feeding had no effect on N utilization (Hoffman et al., 2007).

1.1.3 Carryover effects

The need for further research into the carryover effects of limit-feeding, especially from gestating cows, has been described previously (Driedger and Loerch, 1999). When cows were limit-fed either corn or corn gluten feed during gestation, there was no difference between treatments for BCS at calving (Gunter et al., 2000). Postpartum wt and BCS, as well as those same measurements in lactation, were not different for primiparous (Kruse et al., 2010) or multiparous cows (Winkelman et al., 2007) limit-fed during gestation. Also, when cattle were transferred to ad libitum intake on pasture following limit-feeding, wt and BCS did not differ 60 to 298 d later, giving rise to the notion that there were no deleterious effects of limit-feeding (Gunter et al., 2000). Gestation length was not affected in limit-fed cows (Schoonmaker et al., 2003), but was significantly shorter (2 d) in limit-fed ewes than their counterparts offered high-forage diets (Susin et al., 1995b).

Research has been inconclusive with respect to birth wt from dams limit-fed during gestation. Birth wt of calves did not differ among treatments when dams were limit-fed corn or corn gluten feed with rice hulls or cottonseed hulls (Gunter et al., 2000), corn and alfalfa silage (Kruse et al., 2010), whole shelled corn (Schoonmaker et al., 2003), or silage and corn (Winkelman et al., 2007) compared with calves from cows offered the same diets for ad libitum consumption. Lamb birth wt were not affected by limit-feeding ewes a high-grain (corn-based) diet compared with feeding a high-forage diet for ad libitum consumption. (Susin et al., 1995b).

However, birth wt were 4.2 kg heavier from calves born to cows limit-fed corn compared with their counterparts offered hay for ad libitum consumption in another study (Loerch, 1996). There has been no observance, though, of increased dystocia in either beef (Loerch, 1996; Gunter et al., 2000) or dairy (Hoffman et al., 2007) cows, or beef heifers (Kruse et al., 2010) due to limit-feeding concentrates compared with conventional feeding of hay for ad libitum consumption.

No effects of limit-feeding during gestation on subsequent lactation were observed in dairy cows up to 90 d in milk (Winkelman et al., 2007; Kruse et al., 2010). However, ewes limit-fed during gestation had between 8 and 19% greater milk yields (Susin et al., 1995a). Similarly, no effects on subsequent conception rate were observed with cattle limit-fed the previous winter (Loerch, 1996) or limit-fed during early or late gestation or lactation (Schoonmaker et al., 2003), but limit-fed ewes had a greater percentage conceive in the subsequent breeding period compared with ewes offered an alfalfa cube-based diet for ad libitum consumption (Susin et al., 1995a).

The effects of limit-feeding during gestation on subsequent weaning wt have also been variable. Weaning wt were either 6.6 or 19.7 kg heavier when cows were limit-fed compared with those offered hay for ad libitum consumption (Loerch, 1996), but other studies reported no difference in weaning wt based on gestation diet (Gunter et al., 2000; Schoonmaker et al., 2003).

1.2 Fat effects on digestion and fermentation

Fat has been observed to have detrimental effects on ruminant digestion and metabolism. Fat supplementation from corn oil (64 g/d) resulted in the formation of a fat layer in the rumen, and the rumen contents were white, turbid, and carried a distinct, rotting odor (Brooks et al., 1954). However, fats tended to have less of an adverse effect on digestion and fermentation when hay was included as the main component of the diet (Jenkins, 1993).

Four primary theories of fat effects on digestion have been proposed (Devendra and Lewis, 1974). First, it was posited that dietary fat could actually coat the fiber, thereby inhibiting microbial attachment. Second, the microbial population could be altered through the preferential antimicrobial properties of the fatty acids. Third, microbes may be inhibited through the interactions of dietary fatty acids with cell membranes. Finally, microbes may have less access to necessary cations through the formation of insoluble complexes with long-chain fatty acids. Of the four theories, the first two are accepted as the most plausible and have received the most focus (Jenkins, 1993).

In the "lipid coating" theory, fatty acids would physically adhere to feed particles, especially fiber, and inhibit the close contact necessary for microbial action or block the hydrophilic enzymes, such as cellulases, secreted by the microbes (Jenkins, 1993). Most data, however, seem to support that the actual microbial population is affected rather than their enzymes being inhibited (Palmquist and Jenkins, 1980). One of the potential causes of this would be the cytotoxic effect of uncoupled oxidative phosphorylation (Jenkins, 1993). Lipids may also adhere to lipid bilayer membranes, such as the cell membrane, where they can partition themselves in and disrupt function (Jenkins, 1993). This is especially true of unsaturated fatty acids, which have more toxic effects on rumen microbes than saturated fatty acids (Palmquist and Jenkins, 1980).

1.2.1 Digestion

Most data presented on the inhibitory nature of dietary fat deals with rumen digestive function. Apparent ruminal DM and OM digestibilities were decreased when tallow was added to diets of steers (Boggs et al., 1987). However, post-ruminal OM digestion was improved such that total tract OM digestibility was not altered (Boggs et al., 1987). The lowering of ruminal

digestibility and shift to post-ruminal digestion from adding fat to the diet is a consistent occurrence (Jenkins and Palmquist, 1984; Murphy et al., 1987; Jenkins, 1990; Jenkins and Jenny, 1992).

Fiber appears to be the component most affected by the addition of dietary fat. In vitro cellulose digestibility was reduced as much as 940 g/kg with corn oil addition and in vivo cellulose digestibility was reduced by 520 and 530 g/kg with corn oil and lard additions, respectively (Brooks et al., 1954). Ruminal (Jenkins and Fotouhi, 1990), as well as total tract (Jenkins and Jenny, 1989) ADF digestibilities were affected negatively by added fat. Reduced ruminal fiber digestibility is sometimes compensated with post-ruminal fermentation (Jenkins and Fotouhi, 1990), but the compensation is not sufficient to completely offset the negative impacts on ruminal fiber digestion. Each of these studies however, examined the addition of pure fat to ruminant diets. Results are less conclusive when a feed source with high fat content is added. A nonsignificant trend toward lowered ruminal NDF digestibility was observed when full-fat rapeseed was added to diets, thus mimicking what was observed with DM, but total tract digestibility of NDF was not affected (Murphy et al., 1987). However, ADF digestibility was not affected by the addition of whole cottonseed to the diet (Smith et al., 1981; Keele et al., 1989).

Nitrogen balance was also reduced by added fat. Duodenal flow of microbial N was reduced when tallow was added to steer diets (Boggs et al., 1987). Ruminal and total tract protein digestibility was also reduced with the addition of corn oil to diets (Jenkins and Fotouhi, 1990). However, N intake or absorption in the total tract was not affected when full-fat rapeseed was added to diets (Murphy et al., 1987). Conversely, the addition of prilled fat or canola oil (Jenkins and Jenny, 1992), or the inclusion of whole cottonseed (Smith et al., 1981), has been shown to actually improve N balance. The mechanism for this improvement, in contrast to the

observed decrease in duodenal flow, could be through increasing the efficiency of microbial protein synthesis to accompany the altered dietary protein digestion (Jenkins, 1993)

Also of interest is the impact of dietary fat on overall balance of dietary minerals. Absorption of dietary Ca and Mg were significantly reduced in one study when tallow was offered at 90 g/kg of the total diet (Jenkins and Palmquist, 1984). However, net absorption of Ca, P and Mg were not affected when whole cottonseed was added at 50 to 250 g/kg of the diet (30 to 70 g/kg dietary fat; Smith et al., 1981). Furthermore, absorption of Ca and Mg were not affected, but P absorption was decreased by approximately 7 g/d with the addition of 70 to 140 g/kg dietary fat (Palmquist, 1991).

1.2.2 Fermentation

A strong relationship exists between digestion coefficients of dietary components and the efficiency of conversion of these nutrients to products of fermentation. Addition of tallow to diets (Boggs et al., 1987), or replacing prilled fat with canola oil in ruminant diets (Jenkins and Jenny, 1992) reduced ruminal VFA concentrations. However, total VFA were not affected by the addition of vegetable fat (Chalupa et al., 1986), tallow (Chalupa et al., 1986), prilled fat (Grummer, 1988; Jenkins and Jenny, 1992), hydrogenated fat (Jenkins, 1990), lecithin (Jenkins, 1990; Jenkins and Fotouhi, 1990), or corn oil (Jenkins and Fotouhi, 1990). However, VFA profiles shifted from nonglucogenic to glucogenic with the addition of full-fat rapeseed (Murphy et al., 1987).

In general, acetate as a product of fermentation is not favored by the addition of fat to the diet. Molar percentages of acetate and butyrate were reduced with the addition of tallow (Chalupa et al., 1986; Boggs et al., 1987), vegetable fat (Chalupa et al., 1986), hydrogenated fat (Jenkins, 1990), lecithin (Jenkins, 1990), and yellow grease (Jenkins and Jenny, 1989). In each

case, ruminal propionate concentrations increased, thereby shifting the acetate to propionate ratios in the favor of propionate. This shift to propionate production could be due to a decrease in the ruminal protozoa population with the addition of dietary fats (Keele et al., 1989). The inclusion of prilled fat did not affect ruminal acetate concentrations but increased ruminal propionate concentrations (Grummer, 1988).

Studies reporting the effects of dietary fats on ruminal ammonia-N have yielded inconclusive results. Tallow supplementation did not affect ammonia concentrations (Boggs et al., 1987), but lecithin and corn oil decreased ruminal ammonia-N concentrations, which could lower N loss and improve N retention within the animal (Jenkins and Fotouhi, 1990).

1.2.3 Soap formation

The fourth of the current theories on fat effects on digestion states that microbes may have less access to necessary cations through the formation of insoluble complexes with long-chain fatty acids, also known as ruminal soaps (Devendra and Lewis, 1974). However, the addition of metal cations could reverse the adverse effects of dietary fat inclusion, such as reduced fiber digestibility (Palmquist and Jenkins, 1980).

Soap contents were increased across numerous lengths of time from in vitro digestion with 100 g/kg tallow inclusion, even without additional dietary Ca (Jenkins and Palmquist, 1982). Soap formation was intensified with the addition of dietary Ca sources. It was further noted that solubility was a key factor in soap formation. Calcium chloride was more soluble and resulted in more soap formation than dicalcium phosphate (Jenkins and Palmquist, 1982). This same addition of 100 g/kg dietary fat was observed to more than double the long-chain fatty acid and Ca soap concentrations in vivo (Palmquist et al., 1986). The addition of calcium chloride in this experiment increased the Ca in solution and increased the proportion of long-chain fatty

acids in soaps, but did not increase overall soaps in the rumen. This led the researchers to conclude that an increase in dietary soaps was not as likely to occur with traditional Ca supplements (Palmquist et al., 1986).

It has also been noted that the formation of insoluble fatty acid soaps is directly related to the pH in the rumen (Palmquist et al., 1986). When this concept was explored further, it was noted that common fatty acid soaps of interest in ruminant nutrition (soya, palm fatty acid distillate, tallow and stearic acid) have pKa's between 4.5 and 5.6, meaning that these soaps are fully suited for the rumen environment and would dissociate less than 100 g/kg at physiological pH (Sukhija and Palmquist, 1990). These soaps would then dissociate, though, in the low pH of the abomasum, potentially freeing the cations for absorption by the animal. This range in pKa is also a factor of the types of fats found in insoluble fatty acid soaps. Saturated fats, such as C14:0, C16:0 and C18:0, are more likely to form bonds with soluble cations than unsaturated fatty acids, such as C18:1 and C18:2 (Jenkins and Palmquist, 1982).

The formation of insoluble fatty acid soaps in vivo, or the formation and feeding of soaps, has practical applications within ruminant nutrition. Although corn oil reduced coefficients of digestion in a research study at a time when the mechanism of fatty acid soap formation was not known, it was noted that alfalfa ash was able to counteract these effects (Ward et al., 1957). When rumen inert fats were included in the diet, in situ disappearance of DM and NDF was not affected (Grummer, 1988). Likewise, DM digestibility was not affected when tallow fatty acids, soy fatty acids, or soy soaps were included in the diet, presumably due to the in vivo soap formation by these fatty acids (Jenkins and Palmquist, 1984). There was also no effect on N balance or total ruminal VFA concentration when fat was offered as Ca soaps (Schneider et al., 1988).

1.3 Nitrogen partitioning

Partitioning of N in cattle excreta is of concern with regards to both N use efficiency as well as potential impacts on environmental quality. Of interest in this area is not only the route in which the N-containing compound is excreted, but also the form in which it is excreted.

1.3.1 Route of excretion

Partitioning of N between fecal and urinary output has been an area of interest to researchers for some time. As early as 1941, it was noted that, when fed low-protein basal rations, nearly 930 g/kg of the N excreted by sheep was in the fecal portion compared with urinary excretion (Harris and Mitchell, 1941).

Most differences observed in partitioning of N between urine and feces are a result of the total dietary allowance of N (Mulligan et al., 2004; Yan et al., 2007; Knowlton et al., 2010), although exceptions do occur (Koenig and Beauchemin, 2013b). Approximately 700 g/kg of the surplus N was excreted in the urine of Holstein cows when N surplus was below 150 g/d compared with nearly 1000 g/kg when the surplus exceeded 150 g/d (Bannink et al., 1999). The proportion of total N that was excreted in urine was greater when backgrounding heifers consumed a 140 g/kg CP diet compared with those that consumed a 120 g/kg CP diet (Koenig and Beauchemin, 2013a). A correlation of 0.58 between total N intake and total urinary N excretion was reported (Kertz et al., 1970). This relationship may not be linear however; urinary N increased with N intake, but at a decreasing rate, while the reverse was true for fecal N (Mulligan et al., 2004). When low and high protein diets were compared in another study, an increase in dietary protein increased both fecal and urinary N output (Spek et al., 2013). However, a number of studies have reported that fecal N is generally not affected as greatly, if at

all, by intake or digestibility (Marini and Van Amburgh, 2005; Knowlton et al., 2010; Koenig and Beauchemin, 2013a; Koenig and Beauchemin, 2013b).

Nitrogen excretion is affected by level of feeding, as well. When limit-fed a high concentrate diet, cows excreted 180 g/kg less N, thus making this an environmentally friendly practice (Driedger and Loerch, 1999). Likewise, fecal N excretion decreased linearly with varying degrees of dietary restriction in sheep (Murphy et al., 1994; Susin et al., 1995b), and urinary N excretion decreased with limit-feeding in lambs (Murphy et al., 1994).

1.3.2 Urine components

Of equal importance to N excretion is the form in which the N is eliminated. Nitrogen intake and urinary urea N have been correlated with an r value of 0.50 (Kertz et al., 1970). Across species, urea N has been recorded to account for between 560 and 930 g/kg of the total N excreted in the urine (Bristow et al., 1992). Urea N tends to increase with increasing dietary N intake (Marini and Van Amburgh, 2005; Koenig and Beauchemin, 2013a; Koenig and Beauchemin, 2013b). In fact, with just a 15 g/kg increase in dietary CP, urea N excretion increased by 10 percentage units of the total urinary N (Koenig and Beauchemin, 2013b). Other dietary and physiological factors can influence urea, too. Under conditions of induced acidosis, urine urea N spiked later and decreased later with an increased infusion of glucose into the blood of cattle (Brown et al., 1999). Urea N excretion in the urine has also been noted to decrease in vitamin-A-deficient calves (Woelfel et al., 1963). Degree of corn processing, though hypothesized to potentially affect ruminal microbial N incorporation and, therefore, excretion, had no effect on urinary urea N excretion (Brown et al., 2000). One possible explanation for these observations is that, as dietary N decreases, the efficiency of N use in the body increases (Marini and Van Amburgh, 2005).

When ammonia-N accounted for more than 50 g/kg of the urinary N excretion in goats, researchers attributed this level to hydrolysis that could have occurred between excretion and collection (Bristow et al., 1992). In more recent yr, researchers have given merit to the fact that ammonia-N may be of importance in the characterization of N excretion. While some observed a linear increase in ammonia-N excretion with increasing intake (Marini and Van Amburgh, 2005), most studies have observed no difference in excretion based on dietary or physiological manipulation (Brown et al., 1999; Brown et al., 2000; Koenig and Beauchemin, 2013a; Koenig and Beauchemin, 2013b). It should also be noted that all researchers pointed out that ammonia-N accounted for a small percentage of the total N excreted from the animal.

Purine derivatives are products of purine metabolism and are used as a marker for microbial protein synthesis. Allantoin is the primary purine derivative in livestock excreta, although uric acid, xanthine, hypoxanthine, creatine and creatinine are also of interest (Bristow et al., 1992). Across species (cattle, sheep and goats), allantoin N accounted for up to 118 g/kg of urinary N, uric acid for less than 20 g/kg, the xanthenes for less than 7 g/kg, creatinine N up to 53 g/kg and creatine N up to 63 g/kg, though cattle urine was significantly higher in uric acid compared with sheep and goats (Bristow et al., 1992). In this comparison, Bristow et al. (1992) also observed that creatine was significantly less than creatinine in grazing animals. When fed low protein diets, creatinine N accounted for approximately 250 g/kg of the total urinary N in sheep (Harris and Mitchell, 1941). However, more recent studies have observed no dietary effects on purine derivative excretion, and, moreover, relate purines such as creatinine to body wt rather than diet (Marini and Van Amburgh, 2005).

Urine also contains other minor N-containing components. Hippuric acid has been observed to account for up to 77 g/kg of the total urinary N across species (Bristow et al., 1992).

This compound, has gained interest in recent yrs because of its relation to the conversion of urea to N_2O (van Groenigen et al., 2006). In that study, a 5.1 mmol/kg increase in urine hippuric acid decreased N_2O emissions from the soil by over 500 g/kg and was mainly attributed to the denitrification inhibition of benzoic acid, which is a breakdown product of hippuric acid.

Individual amino acids are also present in small amounts in urine. Glycine is the most abundant of the amino acids in livestock urine, accounting for up to 910 g/kg of the free urinary amino acids in grazing cattle, but as little as 300 g/kg of the free urinary amino acids in cattle consuming concentrate (Bristow et al., 1992). Taurine is second in abundance in urine, and is inversely related to glycine in terms of dietary effect (Bristow et al., 1992).

1.4 Environmental quality

Greenhouse gas emissions have become a major concern of livestock production systems. Such emissions are influenced by variables such as ambient temperature, moisture, aerobic conditions and manure pH (Chianese et al., 2009). Emissions, regardless of type or source, tend to share a positive relationship with temperature, though pH and aerobicity are specific to the pathway and substrate (Chianese et al., 2009).

The major gasses of interest are CO_2, $CH_4$and N_2O. Methane and N_2O have global warming potentials of 23 CO_2-eq/kg and 296 CO_2-eq, respectively (IPCC, 2006). Methane is the major contribution of livestock to greenhouse gas emissions, mostly from enteric fermentation (O'Brien et al., 2009). This is in concert with the fact that, in their stoichiometric relationship, fermentative processes that increase acetate and propionate production (such as manipulations for increased diet digestibility), by default, also increase CH_4 production (Wilkerson et al., 1995). Not only would CH_4 represent an environmental concern that must be addressed (IPCC,

2006), it also represents an energetic inefficiency of the ruminant animal (Ramin and Huhtanen, 2013), which is in direct contradiction to the goal of ruminant nutrition research.

A relatively small contribution (89 kg CO_2-eq/livestock unit yr^{-1}) is due to N_2O (Chianese et al., 2009). Nitrous oxide is an end-product of nitrification and denitrification of the N excreted in livestock waste (US EPA, 2014). While the amount of N in manure is a driving factor in the amount of N_2O potentially emitted, the N must first be removed from potential NH_3 production and nitrified to nitrates or nitrites (US EPA, 2014). Because of this fact, reducing dietary CP has been unsuccessful in changing the N_2O emissions of dairy manure (Lee et al., 2012). Hippuric acid, though, has been shown to decrease the conversion of urine N to N_2O (Bertram et al., 2009).

Cropland tends to be a major sink for C, accounting for an average yearly emission of -8345 kg CO_2/yr (Chianese et al., 2009). This is highly variable, though, and is directly related to the biomass produced (higher for corn and soybean, lower for pasture; Chianese et al., 2009). However, this C is accumulated primarily in the grain, which is then partially returned as CO_2 to the atmosphere when livestock respire following grain consumption. Therefore, the net assimilation of C in cropland biomass is approximately neutral (Chianese et al., 2009).

In a comparison of environmental impact of beef systems over the past 30 yr, Capper (2011) revealed that a combination of shorter birth to slaughter interval and increased wt at slaughter has reduced the energy needed to produce beef. Likewise, feedstuffs use (by wt) by the beef industry was reduced 19% in this time (Capper, 2011). Combining these factors, manure excretion has been reduced by 9.5 billion kg in the United States, N excretion has been reduced by 12%, and P excretion by 10%, resulting in an overall 16% reduction in the United States carbon footprint for beef production (Capper, 2011). Multiple studies have attributed the primary

emission source of beef production to the cow-calf phase (Stackhouse-Lawson et al., 2012; Rotz et al., 2013), and on-farm emissions are generally greater than indirect emissions (O'Brien et al., 2009).

Management system and diet can also play a role in greenhouse gas emission. When use of legumes was compared with feeding of crops fertilized with N, there were no differences in CH_4 emissions, but N_2O emissions were significantly lessened, resulting in an overall 23% decrease in the C footprint of the system (Yan et al., 2012). Also, when dairy systems with and without pasture inclusion were compared, there was a 13% decrease in annual CH_4 emissions when animals were allowed access to pasture (Rotz et al., 2009). This came at the cost of a 33% increase in N_2O contribution, mainly due to high urine N concentrations (Rotz et al., 2009).

Other concerns about the environmental impact of livestock production center around total cycling of N. Nitrogen excretion was increased when cattle were supplemented with additional protein in the form of DDGS (Greenquist et al., 2011) and the majority of the increase was from urinary N. Increasing dietary protein levels has resulted in greater and wetter manure excretions (Frank et al., 2002). Supplementing with corn or wheat DDGS resulted in greater urea and NH_3 excretion as well as increased urinary N excretion (Hünerberg et al., 2013). This increased urinary N excretion is of concern due to its rapid conversion to NH_3, which is not considered a greenhouse gas, but is still of environmental concern (Greenquist et al., 2011; Hünerberg et al., 2013). Because feces and urine are deposited by cattle in different parts of the pasture, as well as different forms, there is a potential for a substantial N loss from urine, especially with urea's rapid hydrolyzation to NH_3 (Rotz, 2004). A reduction in dietary protein can have a great impact on N excreted as well as the potential for environmental N loss. A reduction of 5 percentage units in protein concentration of cattle feed resulted in a 670 g/kg

decrease in NH_3 emissions from sampled manure (Frank et al., 2002). A decrease in N excreta should result in an overall reduction in N loss in the entire cycle (Rotz, 2004).

Also of environmental concern in livestock production is the increased rate of eutrophication of surface waters (Kleinman et al., 2007) related to P runoff (Kleinman et al., 2005). Thus, the form and solubility of manure minerals is also of concern and is a growing area of research (Kleinman et al., 2007). Water-extractable (WE) minerals are known to vary based on animal, diet and manure treatment (Sharpley and Moyer, 2000). Beef manure has been shown to have the lowest mean WEP concentrations of all manures compared, though it did not statistically differ from that of dairy cattle or broilers (Kleinman et al., 2005). When poultry, swine and dairy manures were compared, dairy manure contained the greatest concentration of organic and microbial P (250 and 320 g/kg, respectively; Sharpley and Moyer, 2000). Dairy manure also had 20 percentage units less inorganic P, though 810 g/kg of this was WE and was similar in proportion to poultry manure (Sharpley and Moyer, 2000). Water-extractable minerals also share a commonality with other manure components. Water-extractable P has been shown to have a weak negative correlation to manure DM, and weak positive correlations to WECa and total manure P (Kleinman et al., 2005). This link to manure DM gives rise to the idea that manure water may actually increase manure P solubility (Kleinman et al., 2005). However, comprehensive comparisons of WE minerals, and their relation to availability to livestock production and utilization have yet to be conducted.

2. Rationale

The projects presented in this thesis are intrinsically linked in nature and focus. In Chapter 2, data will be presented in which the efficacy of soybean hulls as a limit-fed co-product feedstuff is evaluated in comparison with hay offered ad libitum to gestating cows. While

previous work has evaluated limit-fed concentrate diets for cows (Loerch, 1996; Driedger and Loerch, 1999; Gunter et al., 2000), none of these studies looked at the potential of soybean hulls as the major dietary ingredient.

Chapter 3 will describe a companion study to Chapter 2 in which ruminally-fistulated cows were used to simulate the dietary constraints of the gestating cows in the first study. Here, unlike past work, the direct effects on total tract digestibility as well as ruminal fermentation and potential environmental impact will be examined using multiple co-products singularly as well as in conjunction with one another. Previous work has described the lack of deleterious effects on limit-fed cows when returned to standard, high-roughage, ad libitum diets (Gunter et al., 2000). However, few, if any, researchers have measured these effects directly. Chapter 4 will describe a portion of the companion study using ruminally-fistulated cows in which in situ techniques were used to determine return to baseline forage ruminal digestibility following a period of limit-feeding.

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CHAPTER 2: PRODUCTION CHARACTERISTICS AND BLOOD METABOLITES OF GESTATING COWS LIMIT-FED SOYBEAN HULLS

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Abstract

Forage for grazing and haying can be limited in drought yr, necessitating that other arrangements be made. The cost of buying and shipping hay often is not justified by its energy density. Co-product feedstuffs, such as soybean hulls (SH), may be a more economical way to maintain a cowherd through such conditions. Our objective was to determine if SH could be used to meet the majority of the energy demands for cows in late gestation. Eighty-six gestating cows (527 ± 7.5 kg initial BW; 4.3 ± 0.27 yr of age) were allocated to 1 of 6 groups on 6 December, 2012. Three of the groups were offered medium-quality bermudagrass hay for ad libitum consumption. The three remaining groups were offered 6.4 kg of SH/cow of daily and allowed access for 1 h daily to a very poor-quality, mixed-grass hay harvested from a Conservation Reserve Program area. Each group was housed in separate 2.02-ha dormant bermudagrass pastures. Cows remained on these treatments for 68 d (until 12 February 2013). Upon calving, birth wt and dystocia scores were recorded, and calves were followed through weaning. Representative bales of each hay were weighed to determine total hay offered. Differences in wt and BCS, and changes in these measurements during the study were minimal between treatments ($P \geq 0.53$). Calf birth wt, weaning wt, wt/d of age at weaning, and birth-to-weaning ADG also

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did not differ ($P \geq 0.11$) between treatments. Based on this information, SH may be limit-fed to cows to meet their energy requirements during late gestation without adverse effects on the cows or their subsequent calves.

1. Introduction

Limit-feeding is a nutritional feeding strategy that is often employed in feedlot diets as "restricted feeding" and in grower programs as "programmed feeding," but is not as commonly employed in cow-calf operations (Galyean, 1999). Previous studies have evaluated the effects of limit-feeding on beef (Loerch, 1996; Gunter et al., 2000; Schoonmaker et al., 2003) or dairy (Hoffman et al., 2007; Winkelman et al.,2007; Kruse et al., 2010) cows. When gestating beef cows were limit-fed corn or corn gluten feed, animals actually exhibited increases in BCS even though BW declined (Gunter et al., 2000). No changes in the rates of dystocia have been recorded when cows were limit-fed (Loerch, 1996; Gunter et al., 2000; Hoffman et al., 2007), and birth wt of calves born to limit-fed cows were either not different (Gunter et al., 2000; Schoonmaker et al., 2003; Kruse et al., 2010) or greater (Loerch, 1996) compared with calves born to cows offered ad libitum forage-based diets. Most of these studies used corn as the limit-fed ingredient, but corn is no longer a cost-effective option. Therefore, our objective was to determine the effect of limit-fed soybean hulls (SH), in conjunction with restricted hay access, on production characteristics of gestating cows.

2. Materials and Methods

2.1 Animals and design

All management and procedures used in this experiment were approved by the Institutional Animal Care and Use Committee at the University of Arkansas (Protocol # 13019). Eighty-six gestating Gelbvieh × Angus cows (527 ± 7.5 kg initial BW; 4.3 ± 0.27 yr of age) were

weighed and BCS assessed (scale of 1 to 9; Whitman, 1975; Spitzer, 1986) on 28 November and 6 December, 2012, and the averages of these measurements were used as the initial values. Cows were stratified by wt within age and allocated randomly to 1 of 6 groups, and each group was housed in a separate 2.02-ha, dormant bermudagrass [*Cynodon dactylon* (L.) Pers.] pasture with negligible forage mass to graze. Groups were then assigned randomly to 1 of 2 treatments. Three groups were offered medium-quality (723 g/kg aNDF, 15 g/kg N) bermudagrass hay, purchased and transported from Mississippi, USA, for ad libitum consumption throughout the study (**HAY**). The remaining three groups were offered 6.4 kg/cow·$d^{-1}$of pelleted soybean hulls (**LSH**). This level was calculated to meet the mean ME requirement, assuming a minimum of 2.3 kg hay consumption daily per cow. Those groups assigned to the LSH treatment were allowed 1 h access each morning to a very poor quality (821 g/kg aNDF, 5 g/kg N) warm-season mixed-grass hay harvested from a Conservation Reserve Program (CRP) area at the Pine Tree Research Station near Pine Tree, AR, USA. Cows remained on their respective treatments for 68 d. Weights were measured and BCS assessed (scale of 1 to 9; Whitman, 1975; Spitzer, 1986) on d 39 and 68 to monitor wt and BCS change. On d 1, 39 and 68, blood was collected via jugular venipuncture (BD Vacutainer® SST™ Plus Blood Collection Tubes, Ref. No. 367985, Becton, Dickson and Co., Franklin Lakes, NJ, USA) from each cow for subsequent analysis for serum non-esterified fatty acids.

Following removal from the treatment diets, cows were co-mingled on bermudagrass pasture. At calving, birth wt and dystocia scores were recorded. Dystocia scores were assigned using the National Assoc. of Anim. Breeders index: 1 = no difficulty; 2 = slight problem; 3 = needed assistance; 4 = considerable force; 5 = extreme difficulty. Calves lost during parturition were considered 5. Calves were weaned from their dams in September, 2013, and managed as a

single herd. Weaning wt, birth-to-weaning ADG and wt/d of age of calves born to cows on the study were also recorded to assess carryover effects of the gestational treatments.

Representative bales of each hay source were selected at random in the first feeding period and weighed to determine average hay bale wt. Hay and SH were sampled at random at the time they were being offered throughout the trial period and composited within the first (d 0-40) or second (d 40-68) half of the study for further analysis. Residual hay and hay waste was estimated visually at the end of the study. This amount was negligible because cows were forced to "clean up" old hay during the final d of the study.

2.2 Chemical analyses and analytical procedures

Representative hay and SH samples were dried to a constant wt at 50°C for DM determination. Representative samples were composited and ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA). Organic matter was determined on all samples via combustion in a muffle furnace (Method 942.05; AOAC, 2000). Neutral detergent fiber (assayed with heat-stable α-amylase and expressed inclusive of residual ash) and ADF (expressed inclusive of residual ash) were measured sequentially using the ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA; Vogel et al., 1999). Lignin (sa) was determined for feed samples using the sulfuric acid method (Method 973.18; AOAC, 2000). Nitrogen was measured using the Dumas total combustion method (Elementar Americas, Mt. Laurel, NJ, USA; Method 990.03; AOAC, 2000).All laboratory analyses were corrected to a DM basis (Method 934.01; AOAC, 2000). Serum non-esterified fatty acids were measured according to the procedure of Johnson and Peters (1993) using a clinical kit (Wako Chemicals USA, Richmond, VA, USA).

2.3 Statistical analyses

Data were analyzed using the mixed models procedure of SAS (SAS Institute, Cary, NC, USA). For purposes of modeling, cows were grouped into three age categories: heifers (2 yr of age; $n = 27$), primiparous cows (3 yr of age; $n = 15$) and multiparous cows (greater than 3 yr of age; $n = 44$). Pastures served as the experimental units in all of the models, and cows (or their calves) were the observational units.

For apparent intake data, the model included the fixed effect treatment, and the random statement included the effect of pasture within treatment. For BW, BCS and serum NEFA concentrations, the model included the fixed effects of treatment, cow age group, d of study, and all possible interactions. Day of study was then used as a repeated measurement with cow as the subject. The random statement included effects of cow within pasture and pasture within treatment. For BCS at calving, dystocia score and calf measurements, the model included the fixed effects of treatment and cow age group. The random statement for BCS at calving and dystocia score included effects of cow within pasture and pasture within treatment, and included the additional terms of calf sex and sire for calf measurements. Means were reported as least squares means for all measurements, and treatments were separated using pairwise *F*-protected *t*-tests. Statistical significance was declared when $P < 0.05$, and a tendency for significance was quantified when $0.05 \leq P \leq 0.10$.

3. Results

Chemical composition of feedstuffs used to evaluate the efficacy of limit-feeding is presented in Table 2.1. The SH used in this study were similar in composition to what would be expected based on published values (NRC, 2000). The hays varied greatly in their respective nutrient composition, with the hay from CRP land that was offered to LSH being of very low

quality. The CRP hay had greater concentrations of aNDF, ADF and lignin (sa) than the hay purchased from Mississippi that was offered to HAY. Additionally, the CRP hay was extremely low in N, having a CP concentration of approximately 31 g/kg.

Apparent hay intake and treatment costs are presented in Table 2.2. By design, cows offered HAY consumed more ($P < 0.01$) DM and OM (g/kg BW) daily from hay compared with LSH. Total apparent daily intake of N (g/kg BW) was greater ($P < 0.01$) from HAY compared with LSH, and total DM (g/kg BW) and aNDF (g/kg BW) apparent daily intake tended to be greater ($P \leq 0.10$) from HAY compared with LSH. Apparent daily OM intake (g/kg BW) did not differ ($P = 0.15$) between treatments. These intake differences, combined with the prices of the different feedstuffs, resulted in a \$0.32/hd·d^{-1} reduction ($P = 0.02$) in feed costs from LSH compared with HAY, culminating in a \$21.55/hd savings over the length of the study.

The 2- and 3-way interactions among treatment, cow age group and d of study did not affect($P \geq 0.12$) cow BW, and only the cow age group × d of study affected ($P = 0.03$) BCS. Likewise, treatment × cow age group interactions did not affect BCS at calving, dystocia scores or calf measurements ($P \geq 0.25$). Therefore, animal production measurements are presented in Table 2.3 by treatment averaged across cow age group, and when appropriate, across dates. Cow average BW and BCS, BCS at calving and dystocia scores did not differ ($P \geq 0.40$) between cows offered the different treatments. Additionally, calf birth wt, weaning wt, age at weaning, wt/d of age and birth-to-weaning ADG did not differ ($P \geq 0.11$) between treatments.

The treatment × cow age group interaction affected ($P = 0.01$) serum NEFA concentrations (Figure 2.1). Serum NEFA concentrations were greater ($P < 0.05$) from heifers receiving LSH than those receiving HAY. However, serum NEFA did not differ ($P \geq 0.12$) between primiparous or multiparous cows offered LSH compared with those offered HAY.

Production measurements are presented by cow age group in Table 2.4. Body wt was greatest ($P < 0.05$) from multiparous cows, followed by primiparous cows and was least from heifers. Weight was also greatest ($P < 0.05$) on d 40, followed by d 60 then d 0 (data not shown). An interaction ($P = 0.03$) of cow age group and day on study was observed for BCS. On d 0, BCS was greater ($P < 0.05$) from multiparous cows than from heifers or primiparous cows, which only tended to differ ($P = 0.09$) from each other. Body condition score did not differ ($P \geq 0.44$) among cow age groups on d 40, but on d 68, BCS was greatest ($P < 0.05$) from multiparous cows, intermediate from primiparous cows and least from heifers. Body condition score at calving, dystocia score, calf birth wt and age at weaning did not differ ($P \geq 0.14$) among cow age groups. Calves born to primiparous cows had lighter ($P < 0.05$) weaning wt compared with heifers or multiparous cows. Calf wt/d of age and birth-to-weaning ADG was greatest ($P < 0.05$) from calves born to heifers, followed by those from multiparous cows, and was least ($P < 0.05$) from those born to primiparous cows.

4. Discussion

By conceptual design of the experiment, limit-fed cows had lower apparent intakes than their counterparts offered ad libitum access to hay. However, the differences in apparent hay intake were not as great as anticipated, as cows assigned to LSH consumed 6.3 kg of hay daily (12 g/kg BW) when we arbitrarily chose a figure of 2.3 kg/d to formulate the amount of SH needed to meet the cow's ME requirements. Based on this rate of consumption in the 1 h of restricted access, it appears that producers may decrease the time of access to less than 1 h. Despite these differences, feeding costs were nonetheless reduced when SH were used for limit-feeding. When ground corn was used as the primary dietary ingredient (Loerch, 1996), a savings of \$0.56 to \$0.77/hd·d^{-1} was realized. However, corn used in the previous study was purchased at

$0.079/kg, which is nearly one fourth of current corn prices. The savings observed in the current experiment are of substantial amount and may justify the use of such a limit-feeding system. With further modifications to the time cows have access to hay, additional savings might be achieved.

Some of the previous studies that evaluated limit-feeding of cows documented increases in BW and BCS when cows were limit-fed (Gunter et al., 2000; Schoonmaker et al., 2003), while others noted loss of BW and BCS (Loerch, 1996). The inconclusive results across previous studies would correctly align with the lack of differences observed in the present experiment. These studies did not compare dietary treatments by age, so little work is available to relate cow age or parity to changes in BW or BCS. The effect of limit-feeding on blood metabolites has been evaluated in dairy cattle (Hoffman et al., 2007; Kruse et al., 2010), but these studies failed to measure NEFA. Limit-fed heifers had greater concentrations of alkaline phosphatase and blood urea nitrogen (Hoffman et al., 2007), but glucose, total protein or albumin were not affected (Hoffman et al., 2007; Kruse et al., 2010). Serum NEFA is a reliable indicator of body condition changes within an animal. An increase in serum NEFA concentration indicates a mobilization of body fat stores or a deficit in energy balance (Bines and Hart, 1982). Since non-esterified fatty acids should increase when cattle are in a negative energy balance, a lack of differences in serum NEFA concentrations between treatments in this study is an indication that LSH did not restrict energy compared with HAY, except in heifers, where the only difference was observed. Thus, limit-feeding may not be a suitable alternative program for heifers.

Birth wt were not affected when cows were limit-fed corn or corn gluten feed (Gunter et al., 2000), corn and alfalfa silage (Kruse et al., 2010), whole shelled corn (Schoonmaker et al., 2003) or silage and corn (Winkelman et al., 2007), or when ewes were limit-fed corn (Susin et

al., 1995), but were greater from cows limit-fed ground corn (Loerch, 1996) when compared with counterparts offered ad libitum. Weaning wt, too, were sometimes greater (Loerch, 1996), but often not different (Gunter et al., 2000; Schoonmaker et al., 2003), from calves born to limit-fed cows in comparison to those fed ad libitum. Results from the current study appear to agree with the majority of the previous studies, with no differences observed between treatments for calf birth wt, weaning wt, or birth-to-weaning ADG.

5. Conclusion

Performance by cows limit-fed soybean hulls was similar to cows allowed ad libitum access to bermudagrass hay in all parameters measured. Body wt and BCS increased for both treatments, and serum NEFA concentrations did not indicate an adverse effect of the limit-feeding strategy when compared with ad libitum hay. Additionally, limit-fed soybean hulls represented a saving of almost $22 per cow over the course of this study. While effects of age were quantified, no effects were observed in calf traits of those born to cows limit-fed soybean hulls in comparison with those offered hay for ad libitum consumption. Therefore, soybean hulls may be limit-fed to cows in mid- to late gestation without adverse effects on cow or calf performance.

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Table 2.1. Chemical analysis of feedstuffs for limit-fed gestating cows

Item[1]	Soybean hulls	Hay (Miss. origin)[2]	Hay (CRP land)
OM, g/kg DM	948	917	927
aNDF, g/kg DM	636	723	821
ADF, g/kg DM	450	382	491
Lignin (sa), g/kg DM	20	35	63
Hemicellulose, g/kg DM	186	341	329
Cellulose, g/kg DM	425	338	419
N, g/kg DM	20	15	5

[1]DM = dry matter; OM = organic matter; aNDF = neutral detergent fiber, heat-stable amylase, inclusive of ash; ADF = acid detergent fiber; lignin (sa) = acid detergent lignin; N = nitrogen.
[2]Bermudagrass hay of Mississippi origin was offered to cows for ad libitum consumption (HAY). Mixed-grass warm-season hay from CRP land was offered to cows under limit-fed conditions (LSH).

Table 2.2. Apparent intake and feed cost for gestating cows offered ad libitum access to hay (HAY) or limit-fed soybean hulls (LSH)

Item[1]	HAY	LSH	SEM[2]	*P*-value[3]
Daily intake as hay				
DM, g/ kg BW	25[a]	11[b]	0.7	< 0.01
OM, g/ kg BW	23[a]	11[b]	0.6	< 0.01
aNDF, g/ kg BW	18[a]	9[b]	0.5	< 0.01
N, g/ kg BW	0.4[a]	0.1[b]	0.01	< 0.01
Total daily intake				
DM, g/ kg BW	25[w]	22[x]	0.7	0.08
OM, g/ kg BW	23	21	0.6	0.15
aNDF, g/ kg BW	18[w]	16[x]	0.5	0.10
N, g/ kg BW	0.4[a]	0.3[b]	0.01	< 0.01
Cost[4]				
$/hd·d^{-1}	2.86[a]	2.54[b]	0.056	0.02
$/hd	194.60[a]	173.05[b]	3.803	0.02

[1]DM = dry matter; OM = organic matter; aNDF = neutral detergent fiber, heat-stable amylase, inclusive of ash; N = nitrogen.
[2]SEM = pooled standard error of the mean.
[3]*P*-values presented are for the main effect of treatment.
[4]Cost of feeding is based on feed costs of $0.24/kg for soybean hulls ($218/ton), $0.20/kg for bermudagrass hay of Mississippi origin ($182/ton) and $0.15/kg for hay from CRP land ($136/ton).
[a,b]Means within a row without a common superscript differ ($P < 0.05$).
[w,x]Means within a row without a common superscript tend to differ ($P < 0.10$).

Table 2.3. Production characteristics of gestating cows offered ad libitum access to hay (HAY) or limit-fed soybean hulls (LSH) and averaged across cow age groups

Item[1]	HAY	LSH	SEM[2]	*P*-value[3]
BW, kg[4]	540	542	9.6	0.91
BCS	6.6	6.6	0.05	0.91
BCS at calving	6.0	5.9	0.12	0.72
Dystocia score[5]	0.0	0.2	0.13	0.40
Calf birth wt, kg	39	43	1.1	0.11
Calf weaning wt, kg	218	215	10.4	0.84
Calf weaning age, d	205	209	2.5	0.36
Calf wt/d of age, kg	1.1	1.0	0.06	0.71
Calf weaning ADG, kg/d	0.9	0.8	0.05	0.60

[1]BCS = body condition score (scale of 1 to 9; Whitman, 1975; Spitzer, 1986); ADG = average daily gain.

[2]SEM = pooled standard error of the mean.

[3]*P*-values presented are for the main effect of treatment since there were no significant interactions between treatment and time ($P \geq 0.12$) or treatment and age group ($P \geq 0.25$),

[4]Cow BW and BCS were averaged across dates since there were no significant interactions between treatment and date ($P \geq 0.12$),

[5]Dystocia scores were assigned using the National Assoc. of Anim. Breeders index: 1 = no difficulty; 2 = slight problem; 3 = needed assistance; 4 = considerable force; 5 = extreme difficulty. Calves lost during parturition were considered 5.

Table 2.4. Production characteristics of gestating cows as influenced by cow age group and averaged across cows offered hay for ad libitum consumption or limit-fed soybean hulls

Item[1]	Heifers (*n* = 27)	Primiparous (*n*=15)	Multiparous (*n* = 44)	SEM[2]	*P*-values[3]
Body wt, kg[4]	491[c]	539[b]	592[a]	10.4	< 0.01
Body condition score					< 0.01
d 0	6.2[b]	6.4[b]	6.7[a]	0.08	
d 40	6.6	6.7	6.6	0.08	
d 68	6.7[b]	6.9[ab]	7.0[a]	0.08	
BCS at calving	5.9	6.0	5.8	0.13	0.69
Dystocia score[5]	0.0	0.0	0.3	0.15	0.32
Calf birth wt, kg	39	43	41	1.2	0.14
Calf weaning wt, kg	236[a]	190[b]	222[a]	8.6	< 0.01
Calf weaning age,d	205	207	210	2.7	0.27
Calf wt/d of age, kg	1.2[a]	0.9[c]	1.1[b]	0.05	< 0.01
Calf ADG, kg/d	1.0[a]	0.7[c]	0.9[b]	0.04	< 0.01

[1]BCS = body condition score (scale of 1 to 9; Whitman, 1975; Spitzer, 1986); ADG = average daily gain.

[2]SEM = pooled standard error of the mean.

[3]Unless presented as means within days, there was no significant interaction of age group and time ($P = 0.85$) or treatment and age group ($P \geq 0.25$). Thus, *P*-values presented are for the main effect of age group.

[4]Cow BW was averaged across dates since no interaction existed between age group and date ($P = 0.83$).

[5]Dystocia scores were assigned using the National Assoc. of Anim. Breeders index: 1 = no difficulty; 2 = slight problem; 3 = needed assistance; 4 = considerable force; 5 = extreme difficulty. Calves lost during parturition were considered 5.

[a,b,c]Means within a row without a common superscript differ ($P < 0.05$).

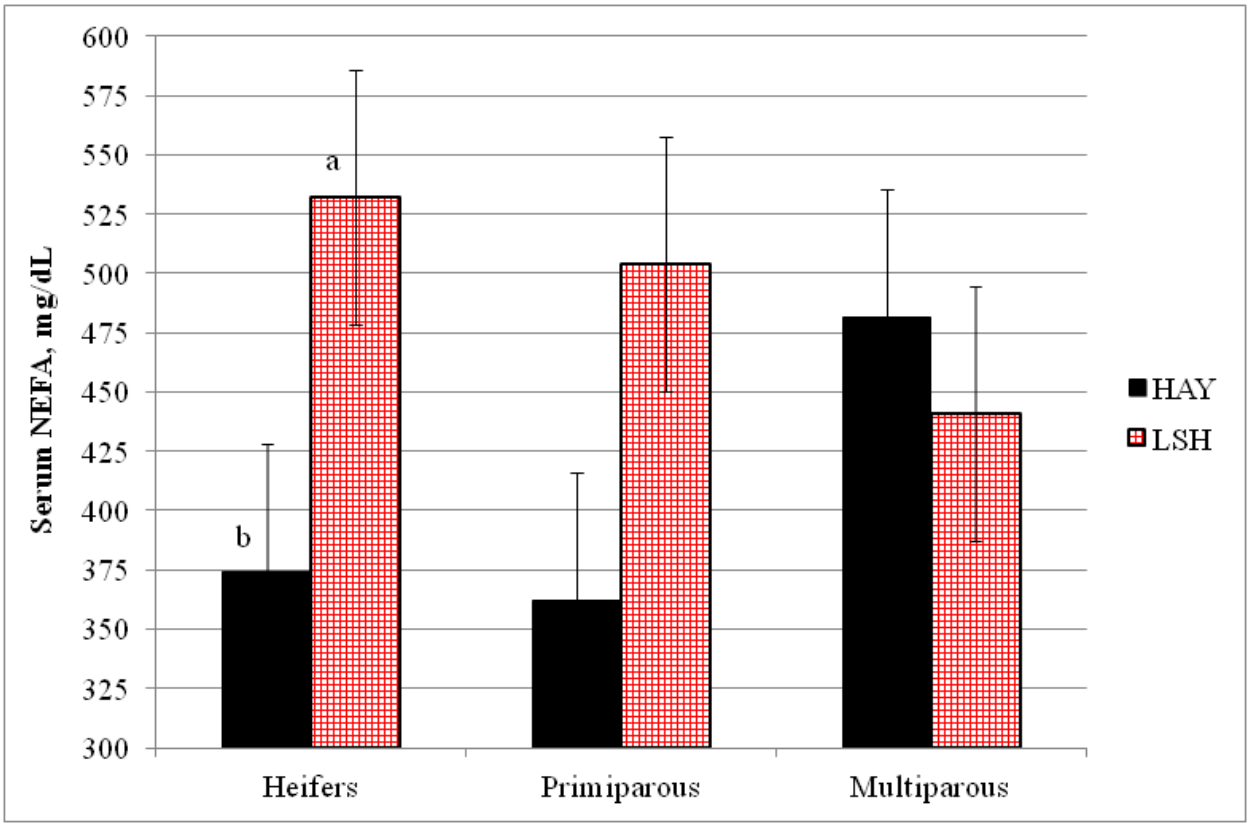

Figure 2.1. Serum non-esterified fatty acid concentrations by treatment and cow age group.
HAY = ad libitum hay; LSH = limit-fed soybean hulls.
Heifers = 2 yr of age (n = 27); primiparous = 3 yr of age (n = 15); multiparous = greater than 3 yr of age (n = 44).
There was a significant interaction of treatment and cow age group (P = 0.01).
[a,b]Means within an age group without a common superscript differ (P < 0.05).

UNIVERSITY OF
ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Kenneth Coffey

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: November 13, 2012

SUBJECT: IACUC Protocol **APPROVAL**
Expiration date : **December 31, 2013**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#13019 - "Performance characteristics and blood metabolites in gestating cows limit-fed rice bran**". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes to the protocol during the research, please notify the IACUC in writing [via the Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **12-31-2013**, you may request an extension (up to 11-12-2015) via Modification Request form. By policy the IACUC cannot approve a study for more than 3 years at atime.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

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DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

UNIVERSITY OF ARKANSAS
DALE BUMPERS COLLEGE
OF AGRICULTURAL, FOOD & LIFE SCIENCES

April 1, 2014

To Whom It May Concern:

I am writing this letter to certify that William Brandon Smith conducted the work and wrote in excess of 51% of chapter 2 of his thesis entitled "*Effect of Limit-Fed Co-product Feedstuffs on Production, Digestion, Fermentation and Rumen Function in Beef Cattle*".

Please contact me if you have further questions or concerns.

Sincerely,

Kenneth P. Coffey, Ph.D.; PAS
Professor, Beef Cattle Nutrition and Management.

ANIMAL SCIENCE DEPARTMENT
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Fayetteville, Arkansas72701
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CHAPTER 3: INTAKE, DIGESTIBILITY AND RUMINAL FERMENTATION CHARACTERISTICS OF COWS LIMIT-FED CO-PRODUCT FEEDSTUFFS

W.B. Smith[1], K.P. Coffey[1], R.T. Rhein[1], E.B. Kegley[1], D. Philipp[1], J.D. Caldwell[2] and A.N. Young[1]

Abstract

Forage for grazing and haying is often limited in droughty yr, requiring other feeding strategies to be implemented. Co-product feedstuffs may provide a more economical way to maintain a cowherd through such conditions. Our objective was to determine the effect of limit-fed co-product feedstuffs on digestive and fermentative characteristics of cows. Eight ruminally-fistulated cows (672 ± 32.0 kg initial BW; approximately 9 yr of age) were stratified by BW and allocated randomly to 1 of 4 diets (2 cows/diet period^{-1}) in a 2-period study: limit-fed soybean hulls (**LSH**), limit-fed distillers' dried grains with solubles (**LDG**), limit-fed an isoenergetic mixture of the two (**MIX**), or provided ad libitum access to hay (**HAY**). Diets were formulated to meet the ME requirements of an 11-month post-partum mature beef cow. Co-product amounts were increased over a 14-d period. This was followed by a 14-d adaptation to diet and facilities and 5 d of total fecal collections. On the final d of fecal collections, rumen fluid was sampled immediately prior to feeding and 2, 4, 6, 8, 10 and 12 hrs post-feeding for measurement of rumen volatile fatty acid and ammonia concentrations. Intake of DM and OM was not different ($P \geq 0.28$) among treatments, but digestibilities of DM, OM, aNDF and ADF were improved ($P < 0.05$) by limit-feeding, and by MIX vs. the mean of LSH and LDG. Total VFA averaged across sampling times were greatest ($P < 0.05$) from LSH, and ruminal ammonia-N was greatest ($P <$

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0.05) from either LDG or MIX at all sampling times. Estimated carbon footprint was not affected ($P \geq 0.16$) by limit-feeding. Therefore, co-product feedstuffs may be limit-fed to cows without negative effects on digestion or ruminal fermentation.

1. Introduction

Limit-feeding, known also as programmed or restricted feeding, is a nutritional strategy employed to avoid overconsumption, decrease manure output or limit pasture use, but is not commonly applied to cow-calf systems (Galyean, 1999). Previous experiments evaluated production aspects of limit-feeding beef (Loerch, 1996; Gunter et al., 2000; Schoonmaker et al., 2003) or dairy cows (Winkelman et al., 2007) or heifers (Hoffman et al., 2007; Kruse et al., 2010), but conducted only limited measurements of digestive function under limit-fed conditions. Limit-fed dairy heifers exhibited no differences in VFA profiles when dietary intake was restricted (Hoffman et al., 2007). Most of these studies used corn as the primary dietary ingredient, but corn is no longer a cost-effective option for limit-feeding. Also, where past work has examined limit-feeding for its environmental incentive of manure reduction (Driedger and Loerch, 1999), the potential carbon footprint reduction of limit-feeding has not been reported. Therefore, our objective was to determine the effect of limit-fed soybean hulls (**SH**), distillers' dried grains with solubles (**DDGS**), a mixture of the two, or ad libitum hay on digestive and fermentative characteristics by cows and subsequent environmental implications.

2. Materials and Methods

2.1 Animals and design

Eight ruminally-fistulated cows (672 ± 32.0 kg initial BW; approximately 9 yr of age) were used in a 2-period experiment with a generalized complete block design to evaluate 4 different diets, where period served as the blocking factor. In each period, cows were stratified

by BW and allocated randomly to 1 of 4diets (2 cows/diet): limit-fed SH (**LSH**), limit-fed DDGS (**LDG**), limit-fed an isoenergetic mixture of SH and DDGS (**MIX**), or provided ad libitum access to mixed-grass warm-season hay (**HAY**). The limit-fed diets were formulated to meet ME requirements of an 11-month post-partum mature beef cow(to best align with cows tested in Chapter 2) based on the published nutritional composition of each feedstuff, and ground limestone was added to the LDG and MIX diets to equalize diet Ca concentrations (NRC, 2000). Cows receiving limit-fed diets were offered 0.9 kg hay daily for roughage consumption. Cows on the HAY diet were offered 0.9 kg of an isoenergetic mixture of SH and DDGS to ensure a non-limiting rumen environment.

At the beginning of each period, cows were offered ad libitum access to hay from large round bales for the first 7 d as a group and were separated each morning at approximately 0800 h and offered increasing levels of their respective supplements. Once their daily supplement amount was reached, the amount of time cows had access to hay was reduced incrementally over the following 7 d. Following this initial adjustment period, cows were moved to an enclosed barn and placed in individual 3.1 × 4.3 m stalls fitted with smooth rubber flooring. Diets were offered at 0800 h daily for a 14-d adaptation period. Cows were allowed a 2-h period to consume concentrates followed by provision of hay as determined by dietary specifications. Orts were collected from feed bunks prior to the 0800 h feeding. Animals had ad libitum access to fresh water throughout the trial, and a trace mineral – salt supplement[3](45 g) was mixed with the concentrate diet daily.

Following the 14-d adaptation period, total feces were collected directly from the pens for

[3]The trace mineral – salt supplement was mixed by adding 900 g/kg fine rock salt (Independent Salt Co., Kanopolis, KS, USA) with 100 g/kg of NB Ruminant Trace Mineral Premix (NB-8675; Nutra Blend, LLC, Neosho, MO, USA) to provide 5 mg/kg Fe, 60 mg/kg Zn, 40 mg/kg Mn, 20 mg/kg Cu, 0.25 mg/kg Co, 1 mg/kg I and 0.3 mg/kg Se.

a 5-d period. Number of visible fecal pats was recorded at 2-h intervals from 0800 to 2000 h daily. For quantification of fecal pats over time, numbers were grouped by time of d. Morning was considered 0800 h to 1200 h, afternoon from 1200 h to 1600 h, evening from 1600 h to 2000 h, and night from 2000 h to 0800 h. At each observation, feces were collected and placed in trash cans lined with plastic can liners. Feces were weighed at 0800 h daily, mixed in a mobile concrete mixer (Kobalt Model 043206, Lowe's LLC, North Wilkesboro, NC, USA), and a subsample taken for chemical analysis. An attempt was made to collect urine once daily from each cow, and the *n* ranged from 1 to 3 per cow and period (with one missing observation). Urine was spot-sampled during natural excretion events via an extendable, hand-held, external collection vessel to minimize disturbance of the animal. Urine pH was measured immediately, and an aliquot of urine was preserved (in duplicate) with 15 g boric acid powder for further analysis. During fecal collections, feeding times of both concentrate and hay were recorded. Time that the components were consumed was recorded to evaluate intake behavior.

On the final d of fecal collections, rumen fluid was sampled immediately prior to feeding and 2, 4, 6, 8, 10 and 12 h post-feeding. Rumen contents were taken from four different regions of the rumen, and fluid was strained through 8 layers of cheesecloth. Rumen fluid pH was measured immediately, and 2 aliquots of rumen fluid were preserved (in duplicate) for further analysis. The first aliquot of 1000 μL was combined with 200 μL of a metaphosphoric acid solution (125 mL/L) containing 2-ethylbutyric acid as an internal standard for subsequent volatile fatty acid analysis. The second aliquot (800 μL) was combined with 400 μL 0.1 *N* HCl for subsequent ammonia-N analysis.

The d following the 5-d collection period, total contents were evacuated from the rumen of each cow immediately prior to feeding. Contents were weighed, mixed thoroughly by hand,

and a subsample taken. Contents were returned to the rumen and cattle fed according to procedure. The following d, total contents of the rumen were evacuated 6-h post-feeding and treated as previously described.

At the end of the first period, cows were co-mingled on a lot of dormant orchardgrass (*Dactylis glomerata* L.) and offered ad libitum access to mixed-grass warm-season hay from large round bales along with 0.9 kg of the isoenergetic mixture of SH and DDGS for 4 weeks. After this period, cows were reallocated randomly to 1 of the 4 diets with the restriction that no cow received the same diet as offered in the first period. The adaptation and collection periods occurred as described previously.

2.2 Chemical analyses and analytical procedures

Feed, ort, feces and ruminal contents were dried to a constant weight at 50°C for DM determination. Representative samples were composited and ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA). Urine samples were frozen upon collection at -20°C, then thawed and composited by cow within period. The composited urine was maintained under refrigeration (2°C).

Organic matter was determined on all samples via combustion in a muffle furnace (Method 942.05; AOAC, 2000). Neutral detergent fiber and ADF were measured sequentially on feed, ort and fecal samples, and aNDF was measured on ruminal contents using the ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA; Vogel et al., 1999). The aNDF procedure included α-amylase and residual ash was not removed. Lignin (sa) was determined for feed samples using the sulfuric acid method (Method 973.18; AOAC, 2000). Acid detergent insoluble ash (ADIA) was measured on feed, orts and dried ruminal content samples by subjecting samples to the ADF procedure, followed by combustion in a

muffle furnace. Ruminal retention time was calculated by using the average fill of ADIA divided by the intake rate/h of ADIA (Waldo et al., 1972).

Nitrogen was measured on feed, ort, fecal and liquid urine samples using the Dumas total combustion method (Elementar Americas, Mt. Laurel, NJ, USA; Method 990.03; AOAC, 2000). Ether extract was measured on feed and ort samples using the Soxhlet extraction method (Method 920.39; AOAC, 2000). All laboratory analyses were corrected to a DM basis (Method 934.01; AOAC, 2000).

Feed, ort and fecal samples were digested in 1.0 M nitric acid and analyzed for P, K, Ca, Mg, S, Na, Fe, Mn, Zn, Cu and B via inductively coupled plasma optical emission spectroscopy (ICP-OES; Method 985.01; AOAC, 2000). Feed, ort and fecal samples were also analyzed for water-extractable minerals and measured via ICP-OES (Kleinman et al., 2007). All minerals measured in urine were assumed to be water-soluble.

Specific gravity of both rumen fluid and urine were determined for calculation of total mass of substances in fluid. Ammonia-N concentrations in preserved rumen fluid and liquid urine were determined colorimetrically (Broderick and Kang, 1980). Additionally, urine samples were analyzed for urea using a clinical kit for blood urea nitrogen (Teco Diagnostics, Anaheim, CA, USA), correcting for endogenous ammonia.

Volatile fatty acids were analyzed by gas liquid chromatography using the method and equipment described in Akins et al. (2009). Total products of fermentation were calculated using VFA concentrations of rumen fluid multiplied by liquid fill. Liquid fill was determined for each cow using the DM determined from evacuated total ruminal contents and the specific gravity of the fluid collected. Dissociative forms of fermentation products were quantified using pH measured at fluid collection and pKa of each of the products by way of the Henderson-

Hasselbalch equation. Bloat potential was determined according to the Akins et al. (2009) modification of Pressey et al. (1963) and Min et al. (2005a, b). Rumen fluid viscosity was determined according to the procedure of Akins et al. (2009).

Carbon dioxide contribution of feeding and respiration were calculated using the predictive equations described by Chianese et al. (2009). Carbon equivalents of the feedstuffs used in the diets were obtained from Adom et al. (2012). Methane emissions were calculated both according to the predictive equations of the IPCC (2006) as well as equation 14b described by Ellis et al. (2007). Nitrous oxide emissions were calculated using IPCC (2006) equations for direct emissions and emissions from volatilization and leaching of N, as well as the equation of Yamulki et al. (1998) for fecal emissions. Carbon footprint was calculated according to the summative approach of the IPCC (2006) using either values obtained from IPCC equations (as well as Chianese et al., 2009; and Adom et al., 2012) or a combination of values obtained from the equations of others (Yamulki et al., 1998; Ellis et al., 2007; Chianese et al., 2009; Adom et al., 2012). Carbon footprint was also expressed without the contribution of respiration or feedstuffs using the principle that CO_2 from respiration is offset by the C sink of crop production (Pitesky et al., 2009).

2.3 Statistical analyses

Intake, digestibility, fill characteristics, nutrient balance and behavioral data were analyzed using the mixed models procedure of SAS® (SAS Institute, Cary, NC, USA). The model included the fixed effect of diet. The random statement included effects of cow and period. Orthogonal contrasts were written to test the effect of HAY in comparison with limit-feeding (average of LSH, LDG and MIX), the effect of SH compared with DDGS (LSH vs. LDG), and the effect of feeding a single co-product feedstuff compared with an isoenergetic

mixture (MIX vs. average of LSH and LDG), and the *F*-test of the contrast was used to detect differences.

Ruminal fermentation characteristics were analyzed using the mixed models procedure of SAS®. The model included the fixed effects of diet, time after feeding and their interaction, and the random effects of cow and period. Time after feeding was used as a repeated measurement with cow as the subject. Diet effects were separated using pairwise *F*-protected *t*-tests within sampling time when the diet × sampling time interaction was detected. All means were reported as least squares means, and statistical significance was declared when $P < 0.05$, and a tendency for significance was quantified when $0.05 \leq P \leq 0.10$.

3. Results

Chemical composition of feedstuffs is presented in Tables 3.1 and 3.2. The SH and DDGS used in this study were similar in composition to what would be expected based on published values (NRC, 2000). Of note is the appreciable concentration of fat in the DDGS (89 g/kg DM). The hay used in this study could be described as medium-quality and is typical of what would be expected of a perennial warm-season hay. Distillers' dried grains with solubles was much greater in concentration of P and S, and much lower in Ca and Fe, in comparison to SH or hay. Additionally, B was undetectable in any feedstuff with the exception of SH.

3.1 Intake, excretion and digestibility

Intake and digestibility of DM and OM are presented in Table 3.3. Contrary to the study design, DM and OM intake (kg/d or g/kg BW) did not differ ($P \geq 0.32$) from HAY compared with the average of the limit-fed diets. Cows offered LSH did, however, tend to consume more ($P = 0.08$) DM (kg/d) compared with those offered LDG. Fecal excretion of DM and OM was greater ($P \leq 0.02$) from cows offered HAY compared with those offered the limit-fed diets

resulting in lower ($P < 0.01$) digestibility of DM and OM from HAY compared with the average of the limit-fed diets. Digestibility of DM and OM was greater ($P \leq 0.03$) from cows offered MIX compared with those offered the single co-products. Digestible DM intake tended to be greater ($P = 0.07$) from cows offered the limit-fed diets compared with those offered HAY, and digestible DM intake tended to be greater ($P = 0.07$) from cows offered LSH compared with those offered LDG. Ether extract intake (g/d, g/kg BW and g/kg DMI) was less ($P \leq 0.05$) from HAY compared with limit-fed diets, and less ($P \leq 0.01$) from LSH compared with LDG.

Intake of aNDF, ADF and hemicellulose (g/kg BW) were greater ($P \leq 0.03$) from cows offered HAY compared with the average of the cows offered the limit-fed treatments, aNDF intake (kg/d) tended to be greater ($P = 0.07$) from HAY compared with the limit-fed treatments, and aNDF and ADF intake was greater (kg/d and g/kg BW; $P \leq 0.02$) from LSH compared with LDG (Table 3.4). Fecal excretions of aNDF, ADF and hemicellulose were greater ($P \leq 0.03$) from HAY than from limit-fed diets. Fecal excretion of ADF was greater ($P = 0.01$) from LSH compared with LDG but the trend was reversed for hemicellulose excretion ($P = 0.05$). Digestibility of aNDF and ADF was less ($P = 0.01$) from HAY compared with the average of the limit-fed diets. Digestibility of aNDF and hemicellulose was greater ($P \leq 0.03$), and digestibility of ADF tended to be greater ($P = 0.07$) from LSH compared with LDG. Digestibility of aNDF and ADF was greater ($P \leq 0.03$) from MIX compared with from the average of the single co-products.

Time to consume the co-product (Table 3.5) offered was less ($P = 0.05$) and time to consume forage greater ($P = 0.01$) from HAY compared with the average of the limit-fed diets. Time to finish the co-product tended to be less ($P = 0.06$) from LSH compared with LDG.

Characteristics of excreta are also presented in Table 3.5. Cows from LSH tended to excrete more ($P = 0.10$) fecal pats in the morning compared with those from LDG. Fecal pat mass (kg/pat) was greater ($P = 0.02$), or tended to be greater (kg DM/pat; $P = 0.06$) from HAY compared with the average of the limit-fed diets. Fecal DM concentrations were less ($P < 0.01$) from HAY compared with the average of the limit-fed diets, and less ($P = 0.01$) from MIX compared with the average of the single co-products. Contrasts were not significant ($P \geq 0.13$) for urine specific gravity (mean = 1.01) or urine solids (mean = 114 g/kg). However, urine pH was greater ($P = 0.01$) from HAY compared with limit-fed diets, greater ($P < 0.01$) from LSH compared with LDG, and greater ($P = 0.02$) from MIX compared with the mean of the single co-products.

Ruminal wet, fluid, DM and OM fill did not exhibit time by diet interactions ($P \geq 0.13$), so main effects will be discussed in table 3.6. Wet, DM and OM fill (g/kg BW) and fluid fill (L) were greater ($P < 0.01$) from HAY compared with the limit-fed diets, and fluid, DM and OM fill were greater ($P \leq 0.01$) from LSH compared with LDG. Fill of DM and OM was less ($P \leq 0.05$) from MIX compared with the mean of LSH and LDG. An interaction of time and diet ($P < 0.05$) was detected for aNDF fill. Ruminal aNDF fill was greatest ($P < 0.05$) from HAY at both sampling times. Among the limit-fed co-product treatments, aNDF fill measured immediately prior to feeding was greater ($P < 0.05$) from LSH compared with MIX, whereas aNDF fill of LDG was intermediate and did not differ compared with LSH and MIX. At 6 h post-feeding, aNDF fill among the limit-fed co-product treatments was greatest ($P < 0.05$) from LSH, then MIX, and least from LDG. Ruminal retention time (h) was greater ($P = 0.01$) from HAY compared iwth the limit-fed diets, and was greater ($P = 0.03$) from LSH compared with LDG.

3.2 Nitrogen balance and partitioning

Nitrogen intake tended to be less ($P = 0.08$) from HAY compared with the limit-fed diets, and was greater ($P = 0.02$) from LDG compared with LSH (Table 3.7). Fecal N concentration was less ($P \leq 0.01$) from HAY compared with the average of the limit-fed diets, and from MIX compared with the average of the single co-products. Fecal N excretion (g/d) also tended to be greater ($P = 0.06$) from HAY compared with the limit-fed diets. Apparent N absorption (g/d and g/kg N intake) was less ($P \leq 0.01$) from HAY compared with limit-fed cows, and less ($P < 0.01$) from LSH compared with LDG. Urine N concentration tended to be less ($P = 0.06$) from LSH compared with LDG.

Urine ammonia-N (mM and g/kg urine N) tended to be less ($P \leq 0.09$) from HAY compared with the limit-fed diets, was less ($P \leq 0.03$) from LSH compared with LDG, and was less ($P = 0.05$) from MIX compared with the single co-products. Urine urea-N (mg/dL) was less ($P = 0.02$) from LSH compared with LDG, but did not differ ($P \geq 0.16$) in any comparison when expressed as g/kg of N.

3.3 Mineral balance

Absorption of minerals is presented in Tables 3.8 through 3.10. Phosphorus consumption (Table 3.8) tended to be less ($P = 0.07$) from HAY compared with the limit-fed diets, and was less ($P < 0.01$) from LSH compared with LDG. Fecal excretion and apparent absorption of P (g/d) were less ($P \leq 0.02$) from LSH compared with LDG, and fecal excretion tended to be less ($P = 0.10$) from HAY compared with the limit-fed diets. Intake and apparent absorption (g/d) of K were greater ($P \leq 0.02$) from HAY than limit-fed diets, and fecal excretion tended to be less ($P = 0.07$), and apparent absorption (g/kg K intake) tended to be more ($P = 0.07$) from MIX compared with the single co-products. Fecal excretion of Ca was less ($P = 0.04$) from MIX

compared with the single co-products. Apparent absorption of Ca (g/kg Ca intake) tended to be greater ($P = 0.06$) from LSH compared with LDG and from MIX compared with the single co-products.

Consumption of Mg (Table 3.9) tended to be greater ($P = 0.07$), and fecal excretion was less ($P = 0.01$) from HAY compared with limit-fed diets. Apparent absorption of Mg (g/kg Mg intake) was greater ($P = 0.01$) from LSH compared with LDG. Sulfur consumption and apparent absorption (g/d and g/kg S intake) were less ($P < 0.01$) from LSH compared with LDG. Apparent absorption of S (g/d and g/kg S intake) tended to be less ($P \leq 0.08$) from HAY compared with the limit-fed diets, and was greater (g/kg S intake; $P = 0.01$) from MIX compared with the mean of the single co-products. Sodium consumption and apparent absorption (g/d) was less ($P \leq 0.04$), and apparent absorption (g/kg Na intake) tended to be less ($P = 0.09$) from HAY compared with the limit-fed diets.

Intake, fecal excretion and apparent absorption (g/d and g/kg Fe intake; Table 3.10) were greater ($P \leq 0.04$) from LSH compared with LDG, and intake tended to be less ($P = 0.06$) from HAY compared with the limit-fed diets. Manganese intake and apparent absorption (mg/d) was greater ($P \leq 0.02$) from HAY compared with the limit-fed diets, and tended to be greater ($P = 0.09$) from LSH compared with LDG. Likewise, Zn intake was greater ($P = 0.04$) from LSH compared with LDG. Copper intake was greater ($P = 0.02$) from LSH compared with LDG, and fecal excretion of Cu tended to be less ($P = 0.10$) from MIX compared with the single co-products.

3.4 Fermentation

The treatment × sampling time interaction affected ($P < 0.05$) ruminal pH (Figure 3.1). Ruminal pH was greatest ($P < 0.05$) from LDG, least from HAY and LSH, and intermediate

from MIX when measured immediately prior to feeding. At 2 h post-feeding, pH was greater ($P < 0.05$) from HAY compared with LDG and MIX. From 4 to 8 h post-feeding, ruminal pH was greater ($P < 0.05$) from HAY compared with MIX and LSH. At 10 h post-feeding, pH tended to be greatest ($P < 0.10$) from HAY and least from LSH, with LDG and MIX intermediate. Ruminal pH did not differ ($P \geq 0.27$) among treatments at 12 h post-feeding.

The treatment × sampling time interaction affected ($P < 0.05$) ruminal ammonia-N concentrations. Ruminal ammonia-N (Figure 3.2) was greatest ($P < 0.05$) from LDG at all sampling times with the exception of 2, 4 and 6 h post-feeding, at which time LDG and MIX did not differ ($P \geq 0.26$). Immediately prior to feeding and 2 and 4 h post-feeding, ammonia-N was lower ($P < 0.05$) from LSH and HAY compared with LDG and MIX. At 4 h post-feeding, ammonia-N concentration was less ($P < 0.05$) from LSH compared with HAY. At 8, 10 and 12 post-feeding, ammonia-N concentrations only tended to differ ($P \geq 0.06$) among HAY, LSH and MIX.

Total ruminal concentrations of VFA (Figure 3.3) were affected by effects of treatment ($P < 0.01$) and time ($P < 0.01$), but not their interaction ($P = 0.93$). However, with the exception of valerate, the treatment × sampling time interaction affected ($P < 0.05$) the individual VFA concentrations along with the acetate:propionate ratio. Total concentrations of VFA were greatest ($P < 0.05$) from LSH, followed by HAY and MIX, and were least from LDG.

Molar concentrations of ruminal acetate (Figure 3.4) were greater ($P < 0.05$) from HAY and LSH compared with LDG at all sampling times. Ruminal acetate concentrations from cows offered MIX were intermediate ($P < 0.05$) between those offered HAY and LSH and those offered LDG at 2 through 6 h post-feeding, but did not differ ($P \geq 0.73$) from HAY and LSH at the other sampling times. Molar concentration of propionate (Table 3.5) followed a reversed

pattern to that of ruminal acetate in that those concentrations were greatest ($P < 0.05$) from LDG at all sampling times. Immediately prior to feeding, molar concentrations of propionate were less ($P < 0.05$) from MIX compared with LSH and HAY. Thus, the acetate:propionate ratio (Figure 3.6) was least ($P < 0.05$) from LDG at all sampling times. The acetate:propionate ratio was greater ($P < 0.05$) from MIX compared with HAY immediately prior to feeding, but lower ($P < 0.05$) compared with LSH at 2, 4, and 6 h after feeding and lower ($P < 0.05$) compared with HAY at 4 h after feeding. Acetate:propionate ratios from HAY, LSH and MIX did not or only tended to differ ($P \geq 0.09$) from each other from 8 through 12 h post-feeding.

Molar percentages of butyrate (Figure 3.7) did not differ ($P \geq 0.12$) among diets immediately prior to feeding, but were greatest ($P < 0.05$) from LDG at all times post-feeding with the exception of at 4 h, at which time LDG tended to be greater ($P = 0.06$) than MIX. Molar percentages of butyrate were not different ($P \geq 0.36$) between HAY and LSH at any of the sampling times, and butyrate concentrations from those treatments were lower ($P < 0.05$) compared with MIX and LDG at 2 through 6 h post-feeding, and lower ($P < 0.05$) compared with LDG at 8, 10,and 12 h post-feeding. Molar percentages of valerate (Figure 3.8) were greatest ($P < 0.05$) from LDG, followed by MIX, LSH and least from HAY.

Molar percentages of isobutyrate (Figure 3.9) immediately prior to feeding were greatest ($P < 0.05$) from LDG followed by MIX. Isobutyrate concentrations were lower ($P < 0.05$) immediately prior to feeding from HAY and LSH than from LDG and MIX. Cows offered LDG had greater ($P < 0.05$) isobutyrate concentrations at 2 and 4 h post-feeding, and tended to have greater ($P < 0.10$) isobutyrate concentrations at 6 h post-feeding compared with cows offered HAY and LSH. Concentrations of isobutyrate from cows offered MIX were intermediate at 2, 4, and 6 h post-feeding and did not or tended to differ ($P \geq 0.09$) from cows offered LDG or HAY.

Concentrations of isobutyrate did not differ ($P \geq 0.13$) among treatments at 8, 10 or 12 h post-feeding. Molar percentages of isovalerate (Figure 3.10) were greatest ($P < 0.05$) from LDG immediately prior to feeding, followed by MIX, then LSH and least from HAY. Isovalerate concentrations were lower ($P < 0.05$) from HAY than LSH at 8 and 10 h after feeding, tended to be lower ($P < 0.10$) from LSH and MIX at 6 h post-feeding, and tended ($P < 0.10$) to be lower than MIX at 4 h post-feeding. Isovalerate concentrations did not differ ($P \geq 0.37$) among treatments at12 h post-feeding. The ratio of straight-chain to branch-chain VFA (Figure 3.11) was not affected by the interaction of time and treatment ($P = 0.14$), but was greatest ($P < 0.05$) from HAY followed by LSH, and least from LDG and MIX.

Total pool of ruminal fermentation products is presented in Table 3.11 by treatment as the interaction of treatment and sampling time did not affect the fermentation pool ($P \geq 0.14$). Ruminal acetate (g) was greater ($P < 0.01$), and propionate (g) tended to be greater ($P = 0.09$) from HAY compared with the average of the limit-fed treatments. Ruminal pool of acetate, butyrate, isovalerate and acetic acid were greater ($P \leq 0.04$), and the pool of isobutyrate, isobutyric acid, butyric acid and isovaleric acid tended to be greater ($P \leq 0.10$), from LSH compared with LDG. Total dissociated products of fermentation were greater ($P \leq 0.01$) from HAY compared with the limit-fed diets, and from LSH compared with LDG. Total products of fermentation were greater ($P < 0.01$) from LSH compared with LDG, and tended to be greater ($P = 0.07$) from HAY compared with the limit-fed diets.

Physical characteristics of rumen fluid are presented in Figures 3.12 through 3.14. Viscosity of rumen fluid did not differ among treatments ($P = 0.27$; Figure 3.12), averaging 8.5 cm traveled in the consistometer, but viscosity appeared to increase ($P = 0.01$) over time (data not shown). Rumen fluid foam height followed no consistent pattern (Figure 3.13). Foam height

of rumen fluid collected immediately prior to feeding tended to be greater ($P < 0.10$) from MIX compared with LDG, and intermediate from HAY and LSH ($P < 0.10$). Foam height of fluid collected 4 h post-feeding was greater ($P < 0.05$) from LSH and HAY compared with that from LDG and MIX. Foam height of fluid collected 8 h post-feeding tended to be greater ($P < 0.10$) from HAY compared with that from MIX, with LSH and LDG intermediate ($P < 0.10$) to the two. Foam strength, measured as the portion of foam remaining after 5 minutes of bubbling CO_2 through the ruminal fluid, was not affected by sampling time or the treatment × sampling time interaction ($P = 0.42$), but was greatest ($P < 0.05$) from LDG, followed LSH and MIX, and least from HAY (Figure 3.14).

3.5 Environmental quality estimates

Water-extractable (WE) minerals are presented in Tables 3.12 and 3.13. Excretion of WEP (g/d and g/kg P excretion; Table 3.12) was less ($P = 0.04$) from HAY compared with the limit-fed diets and was less ($P \leq 0.03$) from LSH compared with LDG. Excretion of WEP (g/d and g/kg P intake) tended to be less ($P \leq 0.09$) from MIX compared with the single co-products. Excretion of WES tended to be less (g/d; $P = 0.09$) from LSH compared with LDG and was greater (g/kg S intake; $P = 0.04$) from HAY compared with the limit-fed diets. Excretion of WENa was greater (g/d; $P = 0.03$) from LSH compared with LDG, and tended to be greater ($P = 0.05$) from HAY compared with the limit-fed diets. Excretion of the WE microminerals was not significant ($P \geq 0.11$) for any comparison (Table 3.13).

Predicted gaseous emissions are presented in Table 3.14. Carbon dioxide from respiration and fuel required in feeding tended to be greater ($P = 0.09$) and the C contribution of feed production tended ($P = 0.07$) to be less from LSH compared with LDG. Carbon dioxide from feed production was less ($P = 0.04$) from HAY compared with the mean of the limit-fed diets.

Estimates of methane production from manure were greater ($P = 0.02$) from HAY compared with the limit-fed diets, and total methane production was greater ($P < 0.01$) from LSH compared with LDG. Direct nitrous oxide emissions, as well as that from volatilization and leaching, tended to be greater ($P = 0.06$) from HAY compared with the limit-fed diets, and fecal nitrous oxide emissions were tended to be greater ($P = 0.06$) from LSH compared with LDG.

Predicted carbon footprint results are presented in Table 3.15. Using the IPCC (2006) summative equation, the CO_2 contribution of feed was less ($P = 0.04$) from HAY compared with the limit-fed diets. Also, the CO_2 contribution of feed tended to be less ($P = 0.07$) and the contribution of respiration and fuel tended to be greater ($P \leq 0.09$) from LSH compared with LDG. The contribution of nitrous oxide tended to be greater ($P = 0.06$) from HAY compared with the limit-fed diets. Using the combined published equations (Yamulki et al., 1998; Ellis et al., 2007; Chianese et al., 2009; and Adom et al., 2012, as well as the conversion factors from IPCC, 2006) in summative form, the CO_2 contribution of respiration and fuel tended to be greater ($P = 0.09$), the contribution of feed tended ($P = 0.07$) to be lower, and the contribution of methane was greater ($P < 0.01$) from LSH compared with LDG, and the CO_2 contribution of feed remained less ($P = 0.04$) from HAY than from the limit-fed diets. The contribution of nitrous oxide tended to be greater (P = 0.06) tended to be greater from LSH compared with LDG. Total C footprint did not differ ($P \geq 0.17$) among comparisons, but total adjusted C footprint was greater ($P < 0.01$) from LSH compared with LDG.

4. Discussion

4.1 Intake, excretion and digestibility

Little explanation can be offered for the discrepancy observed in this study in terms of intake parameters. Since animals were in confinement, intake could have possibly been

hampered. This stands in stark contrast to the observation of limit-fed animals in Chapter 2, in which those offered hay for ad libitum consumption consumed 25 g/kg BW of a hay of similar quality to that offered in this experiment. Because DM intake was not different, interpretation of digestibility parameters may be confounded by type of diet rather than true effects of intake limitation.

One of the seven reasons for instituting a limit-feeding program is the potential of a reduced manure load (Galyean, 1999). When a high-corn diet was offered to cows for limited consumption (29% reduction), a 400 g/kg reduction in DM and OM excretion was realized in comparison to a high-forage diet offered for ad libitum consumption (Driedger and Loerch, 1999). It has also been noted that, in dairy heifers, DM excretion was reduced linearly with decreasing intake (Hoffman et al., 2007), though another study saw no reduction in DM excretion with a 20% reduction in intake (Kruse et al., 2010). The results of the present study are in agreement with the previously published reductions, as cows offered HAY excreted more of all of the components measured compared with cows offered the limit-fed diets.

Dry matter and OM digestibility in the current study were improved when co-products were limit-fed in comparison to HAY, and a positive associative effect was observed for MIX. This is in agreement with previous work (Loerch, 1990; Murphy et al., 1994; Driedger and Loerch, 1999) and has been attributed to the higher energy concentration of the feeds being limit-fed (Galyean et al., 1979), though all of these studies were utilizing corn-based diets and did not examine the potential of co-product feedstuffs. Digestibility of the fiber fractions was less from LDG compared with LSH. Multiple reasons may exist for this reduction in fiber digestion. Inclusion of concentrate in the diet has been shown to decrease fiber digestibility (Mertens and Loften, 1980; Miller and Muntifering, 1985), mainly due to the starch content of the concentrate

ingredients. Dried distillers' grains with solubles, though, also contains a considerable amount of fat (approximately 90 g/kg DM). Fat addition to the diet has been shown to reduce both ruminal (Jenkins and Fotouhi, 1990) and total tract (Brooks et al., 1954; Jenkins and Jenny, 1989) fiber digestibility, though results have demonstrated no reduction when a high-fat feedstuff, such as oilseeds, is added to the diet (Smith et al., 1981; Murphy et al., 1987; Keele et al., 1989).

Cows demonstrated an aversion to consuming DDGS, as noted in both visual observation as well as time to consume concentrate. One possible reason for this may be the method in which diets were formulated. Diets were formulated for an 11-month post-partum and thus, gestating, cow. Since these cows were not in gestation, the diet offered may have been in excess of their requirements, thus causing the cows not to finish all feed offered. Also, it could be that the fat content of DDGS caused a decline in diet palatability. The extended time of consumption with a limit-fed diet is in contrast to previous work with dairy heifers (Hoffman et al., 2007), though others have noted varying feeding behavior in these situations (Hicks et al., 1990).

Time of d in which manure is excreted can potentially affect its environmental impact in terms of emission potential, as emissions are influenced by ambient temperature and moisture (Chianese et al., 2009). As more pats were deposited in the morning from LSH compared with LDG, the emission potential of this manure could be greater, as more moisture would be present on the soil surface at that time, and pats would be freshly deposited as temperatures rose throughout the day. It is likely that nutrients would be more evenly distributed from the limit-fed diets compared with HAY, as the mass of fecal pats from HAY was greater. It is also likely that minerals excreted in the feces from HAY would have a greater runoff potential as feces were wetter from HAY compared with other treatments (Kleinman et al., 2005), most likely due to the water-holding capacity of dietary fiber.

Characteristics of the urine excretion also may play a vital role in assessment of cattle production. Volatilization from LDG, though greater in ammonium concentration, would likely be hampered due to the reduced pH observed in the urine (Chianese et al., 2009), though this reduced urinary pH could be potential for other health issues.

Many studies on limit-feeding have attributed weight loss without the loss of BCS to gastrointestinal fill (Driedger and Loerch, 1999; Gunter et al., 2000; Löest et al., 2001), though it has not been directly measured. This is supported by the current study, in which rumen fill of HAY accounted for up to 44 g/kg BW more than the limit-fed diets. This reduction in gastrointestinal fill has also been used to explain increased efficiency in limit-fed animals (Hicks et al., 1990; Driedger and Loerch, 1999). Some have tried to use passage rate to explain the observed efficiencies of limit-feeding (Murphy et al., 1994; Felix et al., 2011), theorizing that increased residence time would allow for a greater extent of digestion to occur. In the current study, retention time was reduced with limit-feeding (contrary to the previously published hypothesis), even though digestibility was enhanced.

4.2 Nitrogen balance and partitioning

Past work with limit-feeding has generally demonstrated an increase in N absorption and retention with decreased intake, both in cattle (Galyean et al., 1979) and sheep (Murphy et al., 1994). This appears to hold true in the current study, as apparent absorption was improved with limit-feeding. Even so, these data are in contradiction to the notion that limit-feeding can decrease overall N excretion (an environmental incentive) which has been demonstrated previously (Susin et al., 1995; Driedger and Loerch, 1999).

Form of N in excreta also differs based on diet. Even without a significant increase in N intake, urine urea-N concentration was increased in the urine of cows from LDG in the current

study. In general, it has been demonstrated that urea-N excretion increases with increasing N consumption (Marini and Van Amburgh, 2005; Koenig and Beauchemin, 2013). Urine ammonia-N, however, was greater in concentration and proportion of urine N from LDG in the current study. Few researchers have noted increases in urine ammonia-N with increasing intake (Marini and Van Amburgh, 2005), and most have not observed any fluctuations based on experimental treatments (Brown et al., 1999; Brown et al., 2000; Koenig and Beauchemin, 2013).

4.3 Mineral balance

While much attention has been given to diet digestibility and N balance in limit-feeding trials, mineral balance data are lacking in the current literature. Since direct comparisons cannot be made to the literature on limit-feeding, it is more appropriate then to discuss potential reasons for the observations in the current experiment. Consumption of the various minerals was driven by the noted concentrations of these minerals in the feedstuff provided in the diet, and absorption appears to increase with increasing intake.

Surprisingly, apparent absorption of the divalent metal cations was not statistically affected in LDG. When fat is added to the diet, apparent absorption of Ca and Mg tended to be reduced (Jenkins and Palmquist, 1984), mainly due to the formation of insoluble fatty acid soaps (Jenkins and Palmquist, 1982), but this has not necessarily held true when fat was added in the form of oilseeds (Smith et al., 1981). While a characterization of potential soap formation would have been warranted in the current study, laboratory limitations prevented such data collection. Mineral balance data, especially of Ca, yield the conclusion that such soap formation was possible with DDGS inclusion.

Another point of note with DDGS inclusion is the amount of S provided to the animals on a daily basis. The recommended inclusion of S in the diet is less than 3 g/kg DM, with the

maximum tolerable limit of 4 g/kg DM (NRC, 2000), though this assumes an average DMI by the animal and does not take into account a limit-feeding scenario. Excess sulfur in the diet can result in digestive issues and decreased DMI (Loneragan et al., 2001) or in extreme conditions, polioencephalomalacia (PEM; Gould, 1998). Those consuming LDG would have had a dietary S concentration of approximately 8 g/kg DM, double the maximum tolerable limit. Mucous casts were noted in the feces of some of those cows from LDG, indicating gastrointestinal distress. While it is doubtful that animals in this trial were suffering from PEM, increased S intake could potentially explain these occurrences as well as the increased time to finish feed.

4.4 Fermentation

Fermentation, when measured, has not been shown to be affected by limit-feeding (Kruse et al., 2010), but feedstuff variation is known to cause fluctuations. As has been shown previously with concentrate (starch) inclusion in the diet (Wedekind et al., 1986), inclusion of DDGS lowered ruminal pH in comparison to other diets. Distillers' grains inclusion also increased ruminal ammonia as would be expected with increased N intake.

The effect of fat inclusion in the diet on VFA is inconclusive, yielding either an overall reduction (Boggs et al., 1987; Jenkins and Jenny, 1992) or no net change (Chalupa et al., 1986; Grummer, 1988; Jenkins, 1990). A reduction in total VFA concentration was observed with LDG in the current study. It is also known that dietary fat favors propionate over acetate production (Chalupa et al., 1986; Boggs et al., 1987; Jenkins, 1990) in ruminal fermentation, and this was also observed in the present study with cows offered LDG. This has been attributed to a possible decrease in ruminal protozoal counts (Keele et al., 1989), though no information was collected to that effect in the current study. Unlike past work with dietary fat and fermentation, butyrate concentrations were not reduced in the present study with DDGS inclusion.

Unlike most work with ruminal fermentation, the current study presents the products of fermentation not only in terms of concentration, but also total amounts within the rumen. Despite some of the vast differences recorded in VFA concentrations, few translated to total products of fermentation, and propionate was actually greater from HAY despite the concentration differences observed. It may be that this measurement offers more information in terms of actual efficiency of ruminant animals and could be used in future publications to characterize overall effects of dietary components.

Due to previous literature stating a turbidity effect on rumen fluid after the addition of dietary fat (Brooks et al., 1954), it was hypothesized that this may also be true of DDGS inclusion. However, no differences in viscosity were observed. In a characterization of winter wheat types, bloat was positively correlated with in vitro OM digestibility and negatively with aNDF (Akins et al., 2009). In the current study, signs of bloat were observed in LSH, with foam exuding from the cannula. This is supported by the resulting tests performed, indicating that foam production was greatest from LSH 4 h following feeding. Foam strength, though, which would be a true indicator of occurrence of bloat, was less from LSH compared with LDG, even though foam production from LDG was low. This seems to support the previously cited literature in regards to contributing factors for frothy bloat.

4.5 Environmental quality estimates

Water-extractable minerals have been used in environmental applications to characterize the runoff potential of livestock manures (Kleinman et al., 2007), but, as yet, have not been used for application in ruminant nutrition in terms of dietary effects. Using this method, a characterization of WE minerals, especially in relation to intake level, is provided for this experiment. It should be noted that in the original procedure, manures and materials are analyzed

as-is without drying, and, thus, the values provided here can only be used for comparative purposes, as extractability of the minerals is related to manure moisture (Kleinman et al., 2005).

Using the life cycle assessment of various crops, the CO_2-eq contribution of SH would be greater compared with DDGS (Adom et al., 2012), thus leading to an increase in LSH compared with LDG. Though the use of legumes in a cattle production system resulted in no difference in methane emissions (Yan et al., 2012), methane production from LSH was predicted to increase, mainly due to the relative concentrations of ADF and lignin (Ellis et al., 2007). Though hypothesized to yield a lower C footprint, limit-feeding did not affect the relative C contributions in comparison to HAY according to the IPCC calculations (IPCC, 2006), likely due to the reduced amount of feed that would be needed in a limit-feeding system and the efficiency of C incorporation in crop production (Chianese et al., 2009). Using combined summative equations with an adjustment for respiration, however, the single co-products did differ in relative C footprint. It should be noted, though, that the footprints calculated here do not account for urine inputs as urine could not be quantified in the present study.

5. Conclusion

No differences were observed for DM or OM intake, but digestibility of all dietary components was improved with limit-feeding, and apparent absorption of N also tended to be improved with limit-feeding. Limit-feeding co-product feeds did lower rumen pH, but this was not to an extent as to inhibit adequate digestive function. The use of limit-fed soybean hulls greatly increased total VFA, and inclusion of DDGS was observed to increase ruminal concentrations of ammonia-N. Weight loss in limit-feeding schemes may be explained by the increased rumen fill with ad libitum hay. Methane emissions were predicted to be greatest from limit-fed soybean hulls, but limit-feeding did not affect the C footprint of the system. Based on

these data, co-product feedstuffs may be limit-fed to cows without adverse effects on digestive or fermentative function of the rumen.

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Table 3.1. Chemical analysis of feedstuffs used for digestibility measurements in limit-fed cows

Item[1]	Soybean hulls	Distillers' dried grains with solubles	Hay
DM, g/kg	887	892	846
OM, g/kg DM	895	939	883
aNDF, g/kg DM	636	467	712
ADF, g/kg DM	455	139	373
Lignin (sa), g/kg DM	23	19	43
Hemicellulose, g/kg DM	181	328	339
Cellulose, g/kg DM	431	118	319
N, g/kg DM	19	45	18
Ether extract, g/kg DM	16	89	12

[1]DM = dry matter; OM = organic matter; aNDF = neutral detergent fiber, heat-stable amylase, inclusive of ash; ADF = acid detergent fiber, inclusive of ash; Lignin (sa) = acid detergent lignin measured using the sulfuric acid method.

Table 3.2. Mineral composition of feedstuffs used for digestibility measurements in limit-fed cows

Item[1]	SH[2]	DDGS	Hay	TMS	Limestone
Macrominerals					
P, g/kg DM	2	15	4	0	0
K, g/kg DM	17	19	26	2	1
Ca, g/kg DM	11	1	13	27	346
Mg, g/kg DM	4	6	7	1	2
S, g/kg DM	1	8	3	12	3
Na, g/kg DM	2	3	0	83	2
Microminerals					
Fe, mg/kg DM	742	169	488	2229	1349
Mn, mg/kg DM	74	55	151	7738	325
Zn, mg/kg DM	100	109	71	9097	45
Cu, mg/kg DM	18	11	11	4095	15
B, µg/kg DM	311	0	0	0	0
WE Minerals					
WEP, g/kg P	148	184	187	0	1
WEK, g/kg K	260	239	241	81	34
WECa, g/kg Ca	37	468	37	83	1
WEMg, g/kg Mg	90	191	125	78	0
WES, g/kg S	114	163	191	218	2
WENa, g/kg Na	212	203	213	528	204
WEFe, g/kg Fe	69	33	13	2	0
WEMn, g/kg Mn	66	246	39	71	0
WEZn, g/kg Zn	126	167	89	59	17
WECu, g/kg Cu	260	151	315	3	36

[1]DM = dry matter; WE = water-extractable.

[2]SH = soybean hulls; DDGS = distillers' dried grains with solubles; TMS = trace mineralized salt.

Table 3.3. Dry matter and organic matter intake and digestibility and fat intake by cows limit-fed co-product feedstuffs

Item[1]	HAY[2]	LSH	LDG	MIX	SEM[3]	Contrasts[4]
Dry matter						
Intake, kg/d	8.1	9.1	7.0	7.8	0.64	c
Intake, g/kg BW	13.1	13.2	10.5	11.8	1.18	ns
Excretion, kg/d	3.6	2.6	2.1	1.8	0.29	L
Digestion, g/kg DM intake	553	718	696	770	15.2	L, M
Digestible DM intake, g/kg BW	7.0	9.5	7.5	9.0	0.71	l, c
Organic matter						
Intake, kg/d	7.3	8.1	6.4	7.0	0.59	ns
Intake, g/kg BW	11.8	11.8	9.7	10.6	1.00	ns
Excretion, kg/d	2.9	2.2	1.8	1.5	0.24	L
Digestion, g/kg OM intake	609	729	722	785	15.0	L, M
Digestible OM intake, g/kg BW	7.0	8.7	7.2	8.2	0.60	ns
Ether extract						
Intake, g/d	130	145	508	348	66.2	L, C
Intake, g/kg BW	0.2	0.2	0.8	0.6	0.14	L, C
Intake, g/kg DMI	16	16	70	45	7.5	L, C

[1]BW = body weight: DM = dry matter; OM = organic matter; DMI = dry matter intake.
[2]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.
[3]SEM = pooled standard error of the mean.
[4]Contrasts: L, l = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$; 0.10, respectively); C, c = LSH differs from LDG ($P < 0.05$; 0.10, respectively); M = MIX differs from the mean of LSH and LDG ($P < 0.05$); ns = contrasts not significant ($P > 0.10$).

Table 3.4. Fiber intake and digestibility by cows limit-fed co-product feedstuffs

Item[1]	HAY[2]	LSH	LDG	MIX	SEM[3]	Contrasts[4]
Neutral detergent fiber						
Intake, kg/d	5.6	5.8	3.3	4.4	0.40	l, C
Intake, g/kg BW	9.1	8.4	5.0	6.6	0.60	L, C
Excretion, kg/d	2.2	1.6	1.1	1.0	0.19	L
Digestion, g/kg aNDF intake	622	727	658	774	20.4	L, C, M
Digestible aNDF intake, g/kg BW	5.4	6.0	3.5	5.1	0.32	C
Acid detergent fiber						
Intake, kg/d	2.9	4.1	1.1	2.4	0.26	C
Intake, g/kg BW	4.6	5.8	1.9	3.6	0.25	L, C
Excretion, kg/d	1.4	1.3	0.4	0.5	0.13	L, C
Digestion, g/kg ADF intake	533	700	599	771	30.0	L, c, M
Digestible ADF intake, g/kg BW	2.4	4.1	1.2	2.8	0.21	C
Hemicellulose						
Intake, kg/d	2.8	1.7	2.2	1.9	0.18	L
Intake, g/kg BW	4.5	2.5	3.3	3.0	0.38	L
Excretion, kg/d	0.8	0.4	0.7	0.4	0.08	L, C
Digestion, g/kg hemicellulose intake	706	792	684	776	19.9	C
Digestible hemicellulose intake, g/kg BW	3.2	2.0	2.2	2.3	0.22	L

[1]BW = body weight; aNDF = neutral detergent fiber, heat-stable amylase, inclusive of ash; ADF = acid detergent fiber, inclusive of ash.

[2]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[3]SEM = pooled standard error of the mean.

[4]Contrasts: L, l = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$; 0.10, respectively); C, c = LSH differs from LDG ($P < 0.05$; 0.10, respectively); M = MIX differs from the mean of LSH and LDG ($P < 0.05$).

Table 3.5. Intake behavior and excreta characteristics from cows limit-fed co-product feedstuffs

Item	HAY[1]	LSH	LDG	MIX	SEM[2]	Contrasts[3]
Consumption time						
Co-product, h	2.2	9.3	21.4	8.3	4.06	L, c
Forage, h	24.0	5.7	6.6	5.3	4.42	L
Fecal distribution, pats[4]						
Morning	2.0	2.0	1.2	1.8	0.35	c
Afternoon	1.9	1.7	1.7	1.6	0.15	ns
Evening	1.5	1.2	1.3	1.2	0.29	ns
Night	3.1	3.1	2.7	2.9	0.52	ns
Fecal characteristics						
kg/pat	3.1	1.8	1.5	1.5	0.36	L
kg DM/pat	0.5	0.4	0.3	0.3	0.07	l
DM, g/kg	155	198	207	176	7.0	L, M
Urine characteristics						
Specific gravity	1.0	1.0	1.0	1.0	0.00	ns
pH	8.2	8.3	5.7	7.8	0.14	L, C, M
Solids, g/kg	94	118	129	113	29.4	ns

[1] HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[2]SEM = pooled standard error of the mean

[3]Contrasts: L, l = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$; 0.10, respectively); C, c = LSH differs from LDG ($P < 0.05$; 0.10, respectively); M = MIX differs from the mean of LSH and LDG ($P < 0.05$); ns = contrasts not significant ($P > 0.10$).

[4]Morning = 0800 h to 1200 h; Afternoon = 1200 h to 1600 h; Evening = 1600 h to 2000 h; Night = 2000 h to 0800 h.

Table 3.6. Ruminal fill of cows limit-fed co-product feedstuffs

Item[1]	HAY[2]	LSH	LDG	MIX	SEM[3]	Contrasts[4]
Wet fill, g/kg BW	130.7	96.5	86.6	86.6	9.61	L
Fluid fill, L	76.4	59.0	48.4	50.6	4.09	L, C
DM fill, g/kg BW	14.7	10.9	7.6	7.8	0.62	L, C, M
OM fill, g/kg BW	17.0	12.5	8.7	9.0	0.70	L, C, M
aNDF fill, g/kg BW[5]						
Pre-feeding	9.4[a]	5.6[b]	4.4[bc]	3.4[c]	0.53	
6-h post-feeding	14.6[a]	10.4[b]	5.8[d]	7.2[c]	0.53	
Ruminal retention time, h	49.8	32.2	9.3	21.8	4.32	L, C

[1]BW = body weight; DM = dry matter; OM = organic matter; aNDF = neutral detergent fiber, heat-stable amylase, inclusive of ash.

[2]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[3]SEM = pooled standard error of the mean.

[4]Contrasts: L = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$); C = LSH differs from LDG ($P < 0.05$); M = MIX differs from the mean of LSH and LDG ($P < 0.05$).

[5]Interaction of diet and time was significant.

[a,b,c,d]Means within a time without a common superscript letter differ ($P < 0.05$).

Table 3.7. Nitrogen absorption and partitioning from cows limit-fed co-product feedstuffs

Item[1]	HAY[2]	LSH	LDG	MIX	SEM[3]	*n*	Contrasts[4]
N intake, g/d	151	174	282	229	20.8	16	L, C
Fecal N							
g/kg DM	21	26	27	32	1.1	16	L, M
g/d	76	68	55	56	5.7	16	l
Apparent absorption							
g/d	76	106	227	172	18.5	16	L, C
g/kg N intake	504	608	795	749	24.7	16	L, C
Urine NH_3-N							
mM	1	0	92	2	13.0	0.02	l, C, M
g/kg urine N	4	1	87	2	2.6	0.04	l, C, M
Urine urea-N							
mg/dL	139	81	520	143	79.3	0.07	C
g/kg urine N	158	116	339	161	44.9	0.30	ns

[1]DM = dry matter; NH_3 = ammonia.

[2]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[3]SEM = pooled standard error of the mean.

[4]Contrasts: L, l = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$; 0.10, respectively); C = LSH differs from LDG ($P < 0.05$); M = MIX differs from the mean of LSH and LDG ($P < 0.05$); ns = contrasts not significant ($P > 0.10$).

Table 3.8. Phosphorus, potassium and calcium absorption by cows limit-fed co-product feedstuffs

Item	HAY[1]	LSH	LDG	MIX	SEM[2]	*n*	Contrasts[3]
Phosphorus							
Intake, g/d	41	19	93	59	5.9	16	l, C
Fecal excretion, g/d	25	14	50	29	3.1	16	l, C
Apparent absorption							
g/d	15	6	44	29	8.0	16	C
g/kg P intake	409	233	381	436	98.6	16	ns
Potassium							
Intake, g/d	219	162	139	144	18.3	16	L
Fecal excretion, g/d	37	36	35	22	5.8	16	m
Apparent absorption							
g/d	183	126	104	121	16.0	16	L
g/kg K intake	821	794	736	838	31.4	16	m
Calcium							
Intake, g/d	92	97	53	56	14.0	14	ns
Fecal excretion, g/d	79	94	87	47	14.5	14	M
Apparent absorption							
g/d	11	4	-35	10	10.0	14	ns
g/kg Ca intake	131	5	-727	154	111.6	14	c, m

[1]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[2]SEM = pooled standard error of the mean.

[3]Contrasts: l = HAY tends to differ from the mean of LSH, LDG and MIX ($P < 0.10$); C, c = LSH differs from LDG ($P < 0.05$; 0.10, respectively); m = MIX tends to differ from the mean of LSH and LDG ($P < 0.10$); ns = contrasts not significant ($P > 0.10$).

Table 3.9. Magnesium, sulfur and sodium absorption by cows limit-fed co-product feedstuffs

Item	HAY[1]	LSH	LDG	MIX	SEM[2]	*n*	Contrasts[3]
Magnesium							
Intake, g/d	53	36	43	38	4.5	16	l
Fecal excretion, g/d	39	24	32	23	2.6	16	L
Apparent absorption							
g/d	16	14	9	13	3.4	16	ns
g/kg Mg intake	285	365	199	327	62.6	16	C
Sulfur							
Intake, g/d	27	15	50	38	3.2	16	C
Fecal excretion, g/d	12	10	12	10	1.0	16	ns
Apparent absorption							
g/d	14	5	38	24	2.8	16	l, C
g/kg S intake	534	359	751	677	31.2	16	l, C, M
Sodium							
Intake, g/d	2	17	24	14	6.9	14	L
Fecal excretion, g/d	6	12	8	9	1.5	14	ns
Apparent absorption							
g/d	-4	6	15	8	6.1	14	L
g/kg Na intake	-635	-243	600	123	405.8	14	l

[1]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[2]SEM = pooled standard error of the mean.

[3] Contrasts: L, l = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$; 0.10, respectively); C = LSH differs from LDG ($P < 0.05$); M = MIX differs from the mean of LSH and LDG ($P < 0.05$); ns = contrasts not significant ($P > 0.10$).

Table 3.10. Micromineral absorption by cows limit-fed co-product feedstuffs

Item	HAY[1]	LSH	LDG	MIX	SEM[2]	*n*	Contrasts[3]
Iron							
Intake, mg/d	2346	6559	1214	3762	847.5	16	l, C
Fecal excretion, g/d	3962	5881	2354	3288	530.0	16	C
Apparent absorption							
mg/d	-1310	886	-1342	164	684.3	16	C
g/kg Fe intake	-720	204	-1350	0	348.9	16	C
Manganese							
Intake, mg/d	1292	1088	692	590	255.9	16	L, c
Fecal excretion, g/d	1276	1356	1199	1018	159.6	16	ns
Apparent absorption							
mg/d	42	-284	-586	-491	189.3	16	L, c
g/kg Mn intake	-191	-477	-796	-461	270.5	16	ns
Zinc							
Intake, mg/d	855	1299	926	915	216.4	16	C
Fecal excretion, g/d	1180	1891	1562	1330	230.5	16	ns
Apparent absorption							
mg/d	-381	-605	-605	-367	171.7	16	ns
g/kg Zn intake	-499	-575	-674	-401	208.7	16	ns
Copper							
Intake, mg/d	286	333	187	201	74.2	16	C
Fecal excretion, g/d	314	528	421	348	61.1	16	m
Apparent absorption							
mg/d	-107	-196	-217	-134	68.8	16	ns
g/kg Cu intake	-523	-745	-1280	-1600	818.3	16	ns

[1]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[2]SEM = pooled standard error of the mean.

[3] Contrasts: l = HAY tends to differ from the mean of LSH, LDG and MIX ($P < 0.10$); C, c = LSH differs from LDG ($P < 0.05$; 0.10, respectively); m = MIX tends to differ from the mean of LSH and LDG ($P < 0.10$); ns = contrasts not significant ($P > 0.10$).

Table 3.11. Ruminal fermentation pool, averaged across sampling times, from cows limit-fed co-product feedstuffs[1]

Item	HAY[2]	LSH	LDG	MIX	SEM[3]	Contrasts[4]
Dissociated products of fermentation[5]						
Ammonia, g	0.1	0.0	0.1	0.0	0.02	ns
Acetate, g	225.4	201.7	64.7	119.8	80.31	L, C
Propionate, g	72.6	66.5	50.0	45.6	14.23	l
Isobutyrate, g	4.5	4.3	3.0	3.8	0.87	c
Butyrate, g	36.6	36.3	21.3	27.6	7.32	C
Isovalerate, g	5.7	8.4	5.0	7.0	1.49	C
Valerate, g	4.4	5.2	6.1	4.4	1.16	ns
Total, g	349.8	322.7	150.1	207.9	53.91	L, C
Undissociated products of fermentation						
Ammonium, g	11.6	6.3	13.8	10.1	2.10	C
Acetic acid, g	2.8	26.8	4.1	7.0	7.71	C
Propionic acid, g	1.2	12.5	4.8	3.8	3.99	ns
Isobutyric acid, g	0.1	0.6	0.2	0.2	0.19	c
Butyric acid, g	0.6	5.6	1.7	2.1	1.79	c
Isovaleric acid, g	0.1	1.1	0.1	0.3	0.41	c
Valeric acid, g	0.1	0.9	0.5	0.3	0.29	ns
Total, g	17.6	54.3	24.4	23.5	15.10	ns
Total products of fermentation						
g	368.1	377.1	174.7	231.3	65.68	l, C

[1]Amounts in the fermentation pool were derived from liquid concentrations of the products of fermentation multiplied by the total volume of rumen fluid assessed during total rumen evacuations (total mass minus dry matter mass multiplied by the rumen fluid density).

[2]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[3]SEM = pooled standard error of the mean.

[4]Contrasts: L, l = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$; 0.10, respectively); C, c = LSH differs from LDG ($P < 0.05$; 0.10, respectively); ns = contrasts not significant ($P > 0.10$).

[5]Dissociated and undissociated products of fermentation were determined using the pH of rumen fluid at the time points of total rumen evacuations and the pKa of the volatile fatty acids and ammonia in the Henderson-Hasselbalch equation.

Table 3.12. Water-extractable (WE) macromineral fecal excretion by cows limit-fed co-product feedstuffs

Item	HAY[1]	LSH	LDG	MIX	SEM[2]	*n*	Contrasts[3]
Phosphorus							
g/d	0.8	0.8	4.1	1.4	0.55	16	L, C, m
g/kg P excretion	34	48	83	52	13.9	16	L, C
g/kg P intake	21	40	45	26	8.3	16	m
Potassium							
g/d	7.8	9.3	7.0	6.3	1.98	16	ns
g/kg K excretion	240	257	183	290	40.9	16	ns
g/kg K intake	38	57	51	43	11.3	16	ns
Calcium							
g/d	1.8	1.9	0.9	0.7	0.43	16	ns
g/kg Ca excretion	242	227	131	160	6.3	16	ns
g/kg Ca intake	22	23	28	15		14	ns
Magnesium							
g/d	2.5	2.3	3.0	1.8	0.47	16	ns
g/kg Mg excretion	67	92	93	81	17.7	16	ns
g/kg Mg intake	50	70	65	53	13.6	16	ns
Sulfur							
g/d	0.6	0.6	1.3	0.8	0.21	16	c
g/kg S excretion	55	65	99	87	15.9	16	ns
g/kg S intake	27	43	22	28	6.5	16	C
Sodium							
g/d	2.1	2.7	0.9	2.0	0.48	16	C
g/kg Na excretion	221	216	147	252	35.2	16	ns
g/kg Na intake	314	249	110	224	83.2	14	l

[1]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[2]SEM = pooled standard error of the mean.

[3]Contrasts: L, l = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$; 0.10, respectively); C, c = LSH differs from LDG ($P < 0.05$; 0.10, respectively); m = MIX tends to differ from the mean of LSH and LDG ($P < 0.10$); ns = contrasts not significant ($P > 0.10$).

Table 3.13. Water-extractable (WE) micromineral fecal excretion by cows limit-fed co-product feedstuffs

Item	HAY[1]	LSH	LDG	MIX	SEM[2]	*n*	Contrasts[3]
Iron							
mg/d	38.8	29.0	16.5	18.3	7.60	16	ns
g/kg Fe excretion	12	5	7	6	3.4	16	ns
g/kg Fe intake	18	5	15	5	4.4	16	ns
Manganese							
mg/d	6.5	6.0	6.8	3.5	2.01	16	ns
g/kg Mn excretion	5	5	6	3	1.9	16	ns
g/kg Mn intake	7	9	9	5	3.4	16	ns
Zinc							
mg/d	11.7	17.6	12.0	11.2	2.69	16	ns
g/kg Zn excretion	10	11	7	9	1.5	16	ns
g/kg Zn intake	15	15	13	11	2.4	16	ns
Copper							
mg/d	14.9	17.2	10.5	9.4	3.14	16	ns
g/kg Cu excretion	44	30	26	31	7.3	16	ns
g/kg Cu intake	66	53	59	79	26.5	16	ns

[1]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.
[2]SEM = pooled standard error of the mean.
[3]Contrasts: ns = contrasts not significant ($P > 0.10$).

Table 3.14. Predicted gaseous emissions from cows limit-fed co-product feedstuffs

Item	HAY[1]	LSH	LDG	MIX	SEM[2]	Contrasts[3]
Carbon dioxide						
Respiration, kg/d[*]	8	8	7	7	0.7	c
Fuel, g/d[*]	323	324	300	308	29.0	c
Feed prod., kg/d[**]	4.0	4.7	6.1	5.3	0.39	L, c
Methane						
Enteric, g/d[†]	32	41	35	34	3.3	ns
Manure, g/d[†]	6	4	3	3	0.5	L
Total, g/d[††]	103	169	83	120	11.3	C
Nitrous oxide						
Direct, mg/d[†]	2404	2143	1717	1772	178.7	l
Volatilization, mg/d[†]	360	322	258	266	26.8	l
N Leaching, mg/d[†]	90	80	65	66	6.7	l
Fecal, mg/d[‡]	313	342	268	341	26.4	c

[1]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[2]SEM = pooled standard error of the mean.

[3]Contrasts: L, l = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$; 0.10, respectively); C, c = LSH differs from LDG ($P < 0.05$; 0.10, respectively); ns = contrasts not significant ($P > 0.10$).

[*] Calculated according to the equations of Chianese et al., 2009.

[**]Calculated according to the equations of Adom et al., 2012.

[†] Calculated according to the equations of IPCC, 2006.

[††]Calculated according to the equations of Ellis et al., 2007.

[‡] Calculated according to the equations of Yamulki et al., 1998.

Table 3.15. Predicted carbon footprint of cows limit-fed co-product feedstuffs

Item	HAY[1]	LSH	LDG	MIX	SEM[2]	Contrasts[3]
IPCC carbon footprint[*], kg CO_2-eq/d						
Feed	4.0	4.7	6.1	5.3	0.39	L, c
Respiration	7.7	7.7	7.1	7.3	0.69	c
Fuel	0.32	0.32	0.30	0.31	0.029	c
Methane	0.7	0.9	0.9	1.0	0.15	ns
Nitrous oxide	0.9	0.8	0.6	0.6	0.06	l
Total	13.3	14.5	15.1	14.4	0.97	ns
Carbon footprint from combined equations[**], kg CO_2-eq/d						
Feed	4.0	4.7	6.1	5.3	0.39	L, c
Respiration	7.7	7.7	7.1	7.3	0.69	c
Fuel	0.32	0.32	0.30	0.31	0.029	c
Methane	2.4	3.9	1.9	2.8	0.26	C
Nitrous oxide	0.09	0.10	0.08	0.10	0.008	c
Total	14.1	16.8	15.7	15.6	1.17	ns
Adjusted total[†]	2.7	4.3	2.3	3.2	0.29	C

[1]HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles.

[2]SEM = pooled standard error of the mean.

[3]Contrasts: L = HAY differs from the mean of LSH, LDG and MIX ($P < 0.05$); C, c = LSH differs from LDG ($P < 0.05$; 0.10, respectively); ns = contrasts not significant ($P > 0.10$).

[*] Calculated according to the equations of IPCC, 2006, with values from Chianese et al., 2009, and Adom et al., 2012.

[**]Calculated according to the combined equations of Yamulki et al., 1998; Ellis et al., 2007; Chianese et al., 2009; and Adom et al., 2012, as well as the conversion factors from IPCC, 2006.

[†] The adjusted total C footprint takes into account the principle of Pitesky et al. (2009) that the contribution of respiration is offset by the sink of crop production.

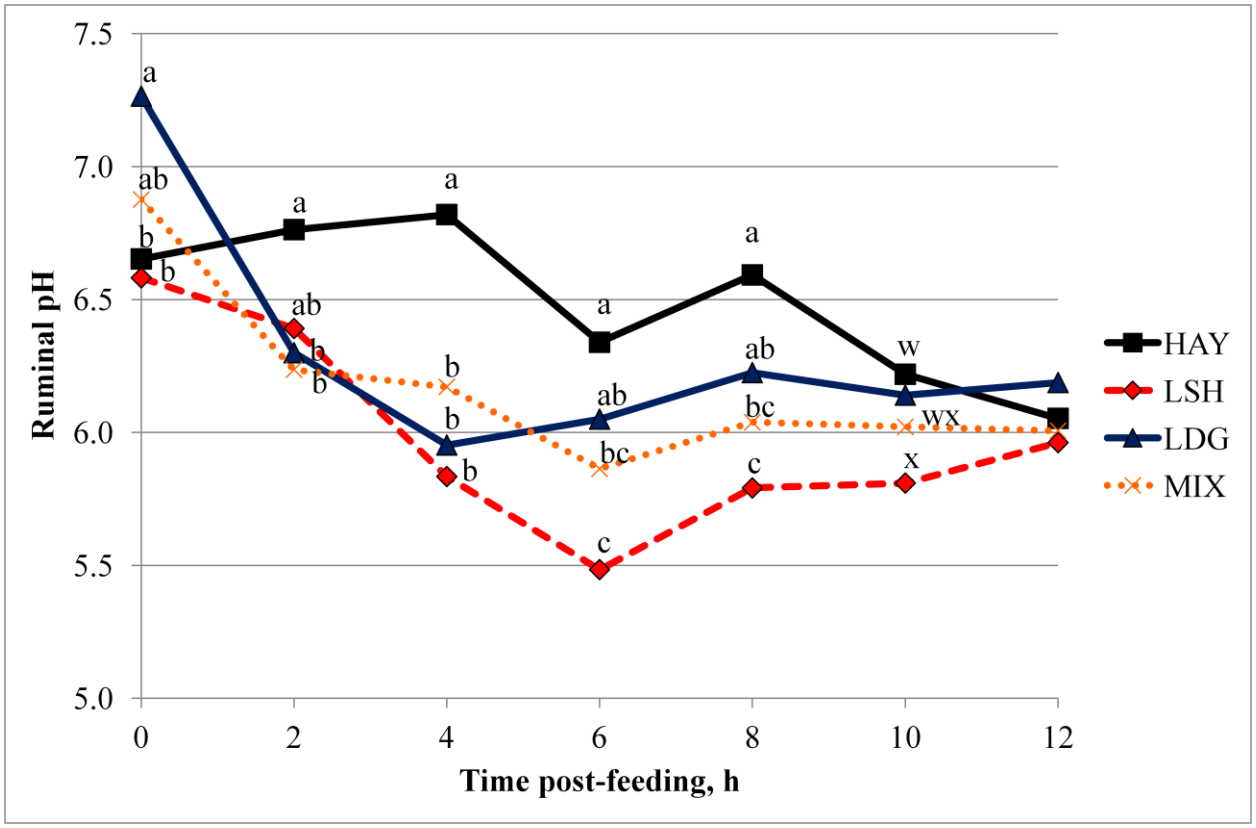

Figure 3.1. Ruminal pH over time after feeding for cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal pH varied by diet ($P < 0.01$), time ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time without a common superscript differ ($P < 0.05$).
[w,x,y]Means within a time without a common superscript tend to differ ($0.05 \leq P \leq 0.10$).

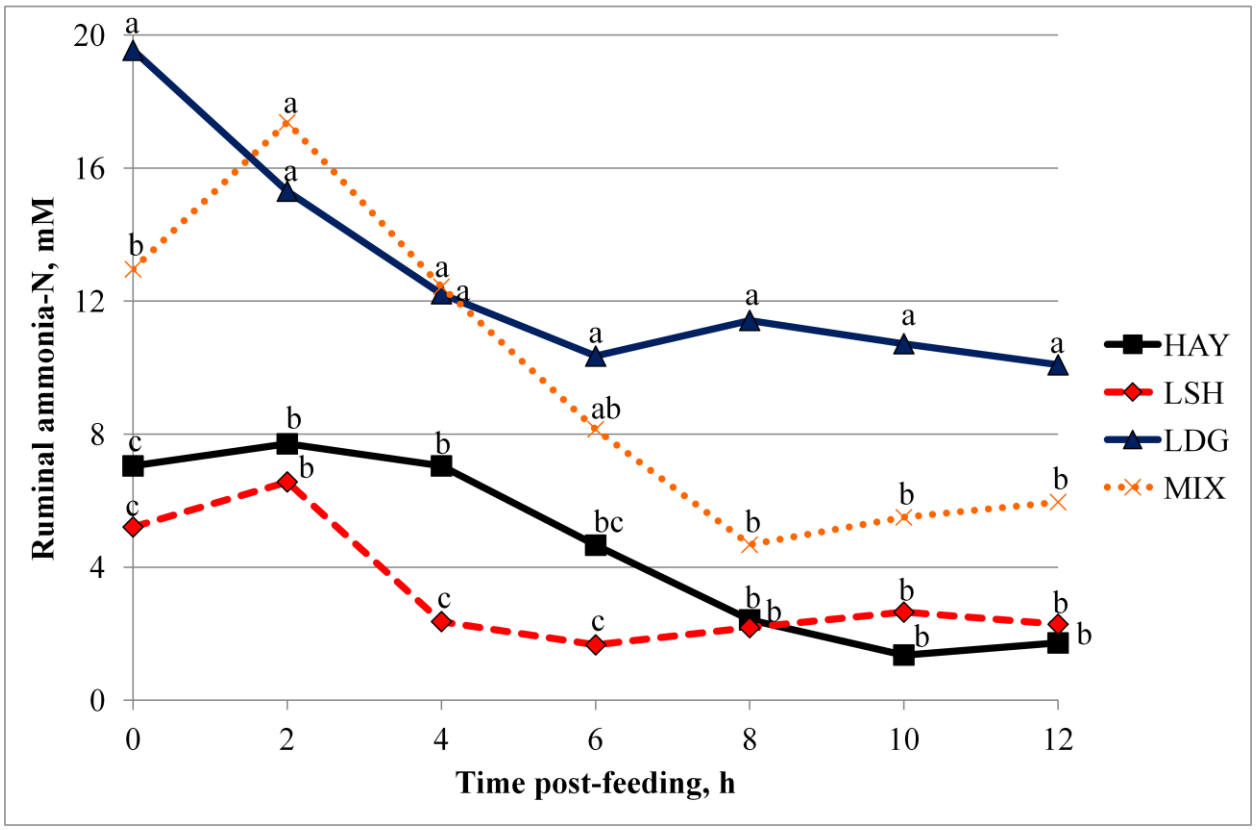

Figure 3.2. Ruminal ammonia nitrogen (mM) over time after feeding for cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal ammonia N varied by diet ($P < 0.01$), time ($P < 0.01$), and their interaction ($P = 0.02$).
[a,b,c]Means within a time without a common superscript differ ($P < 0.05$).

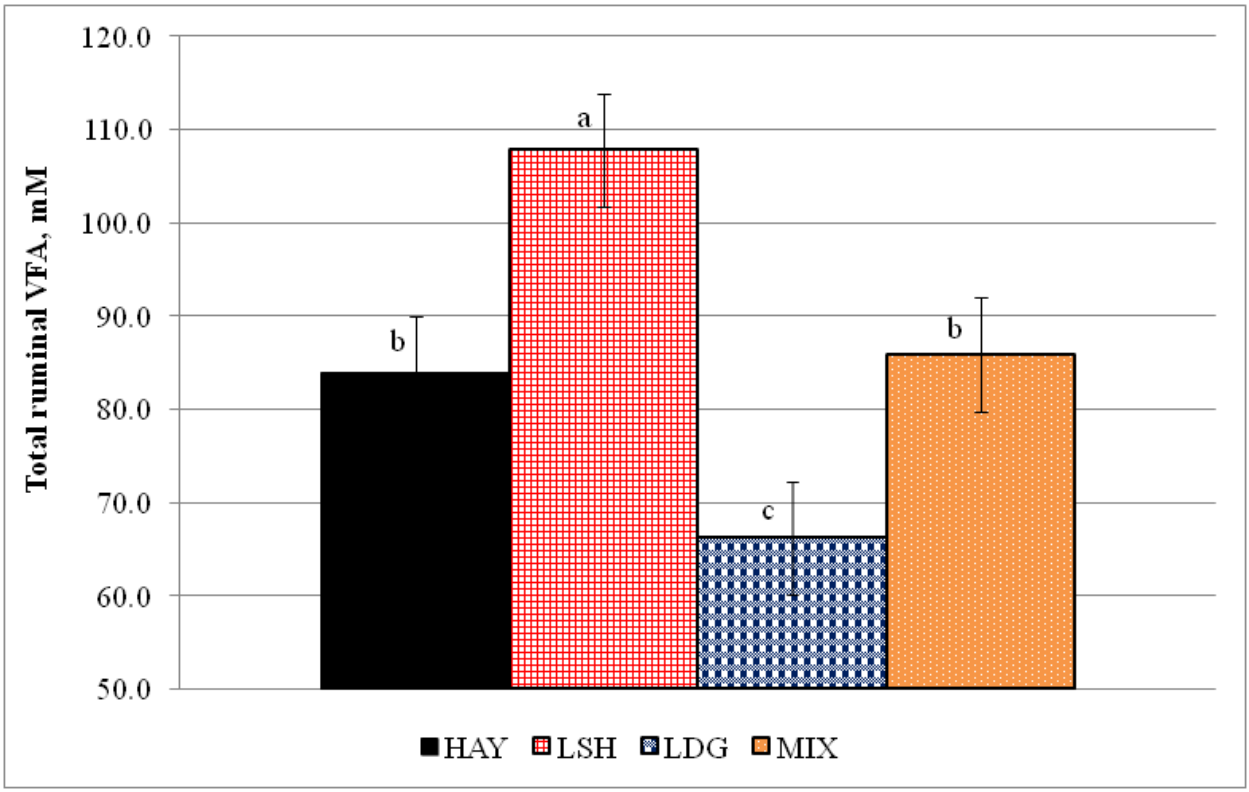

Figure 3.3. Ruminal total volatile fatty acid (VFA) concentrations (mM) averaged across sampling times from cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal total VFA varied by diet ($P < 0.01$) and time ($P < 0.01$), but not their interaction ($P = 0.93$).
[a,b,c]Means without a common superscript differ ($P < 0.05$).

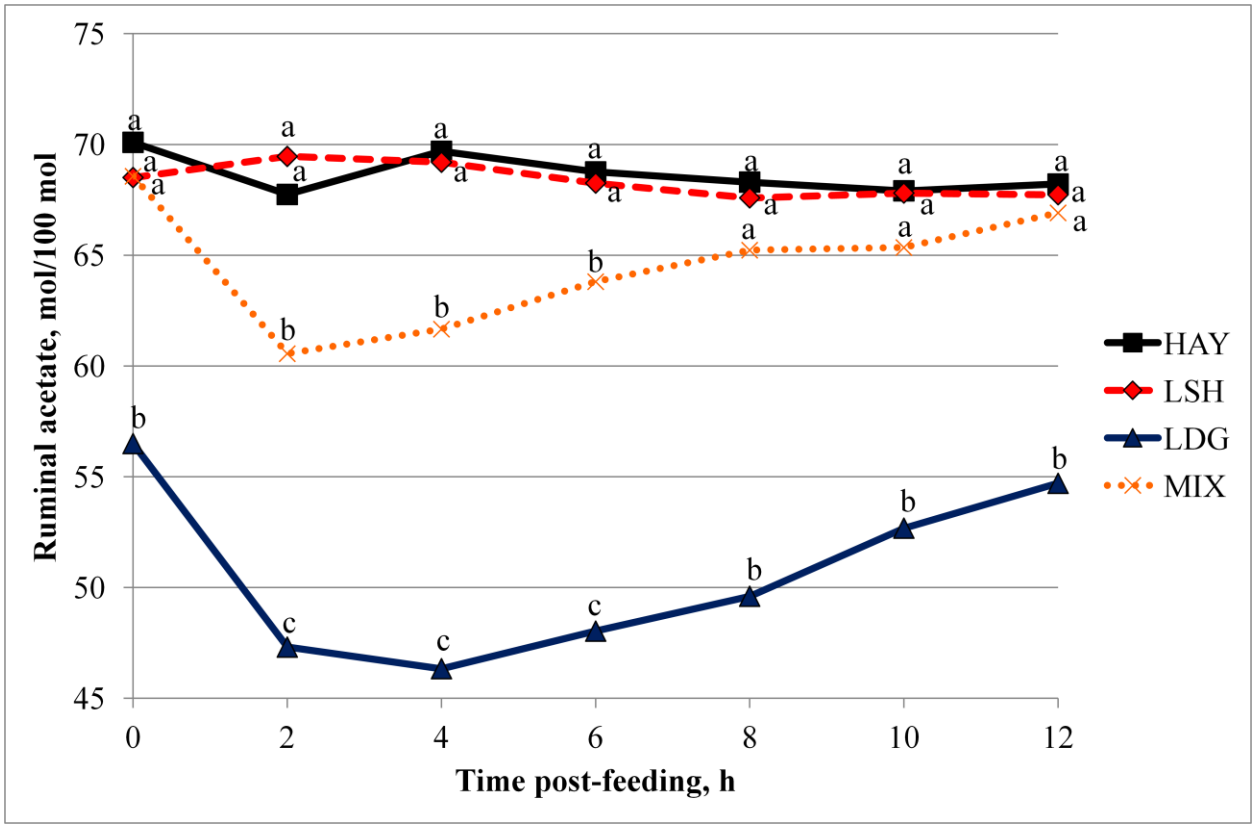

Figure 3.4. Ruminal acetate concentrations (mole/100 mole)over time after feeding for cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal acetate concentrations varied by diet ($P < 0.01$), time ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time without a common superscript differ ($P < 0.05$).

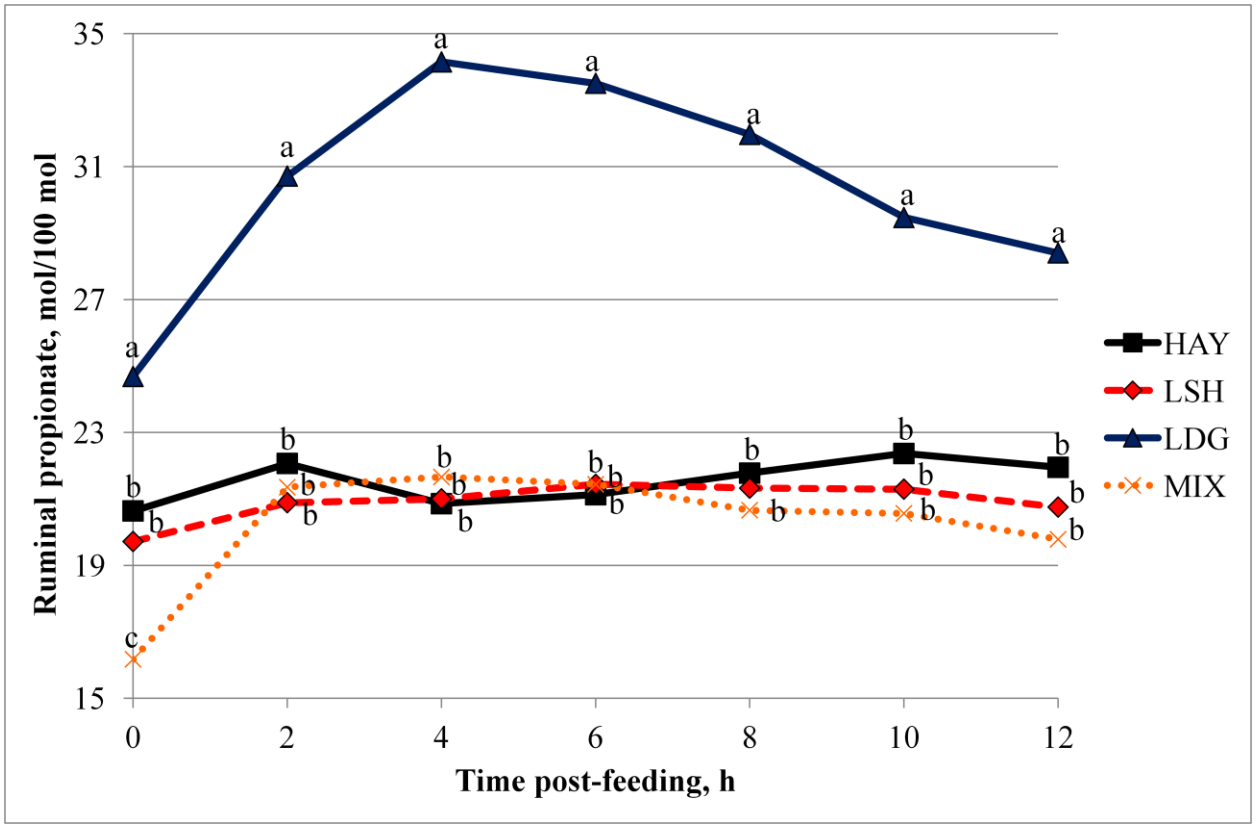

Figure 3.5. Ruminal propionate concentrations (mole/100 mole) over time after feeding for cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal propionate concentrations varied by diet ($P < 0.01$), time ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time without a common superscript differ ($P < 0.05$).

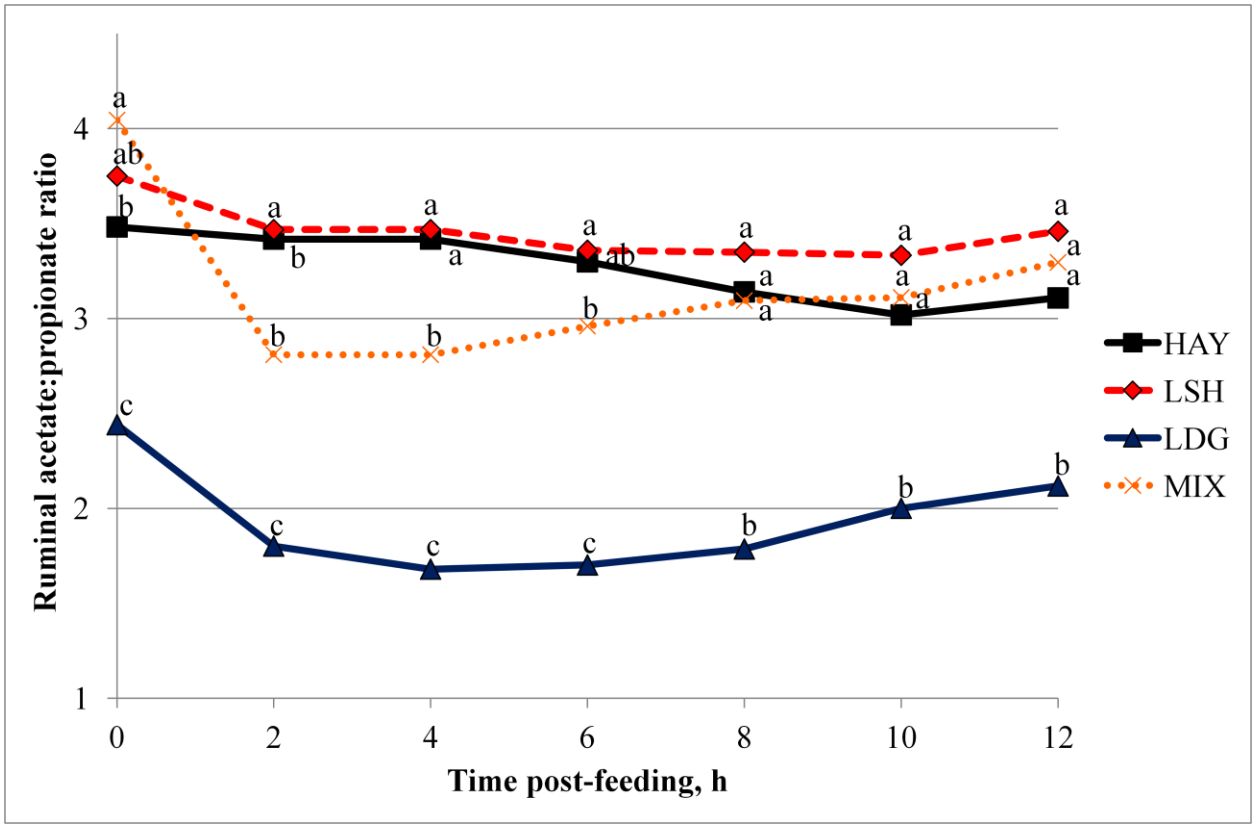

Figure 3.6. Ruminal acetate:propionate ratio over time after feeding for cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal acetate:propionate ratio varied by diet ($P < 0.01$), time ($P < 0.01$), and their interaction ($P = 0.01$).
[a,b,c]Means within a time without a common superscript differ ($P < 0.05$).

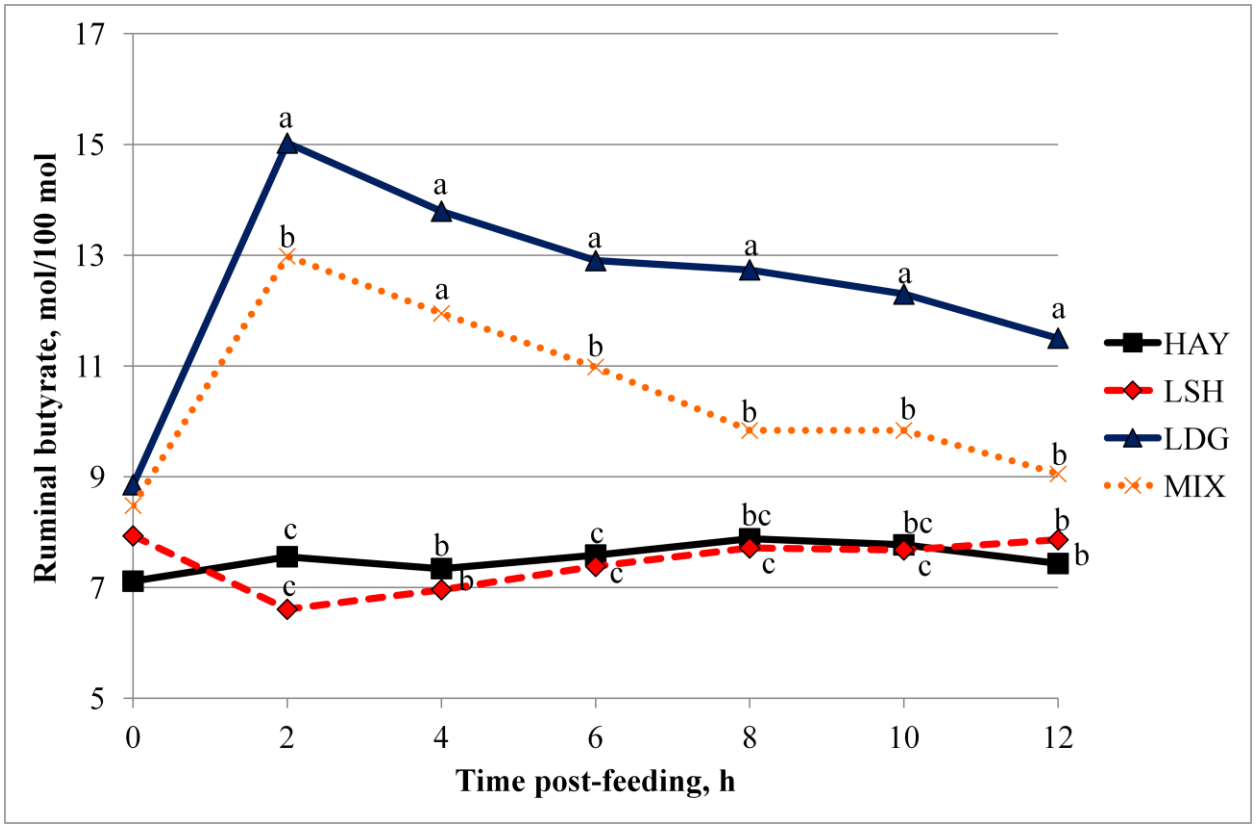

Figure 3.7. Ruminal butyrate concentrations (mole/100 mole) over time after feeding for cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal butyrate concentrations varied by diet ($P < 0.01$), time ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time without a common superscript differ ($P < 0.05$).

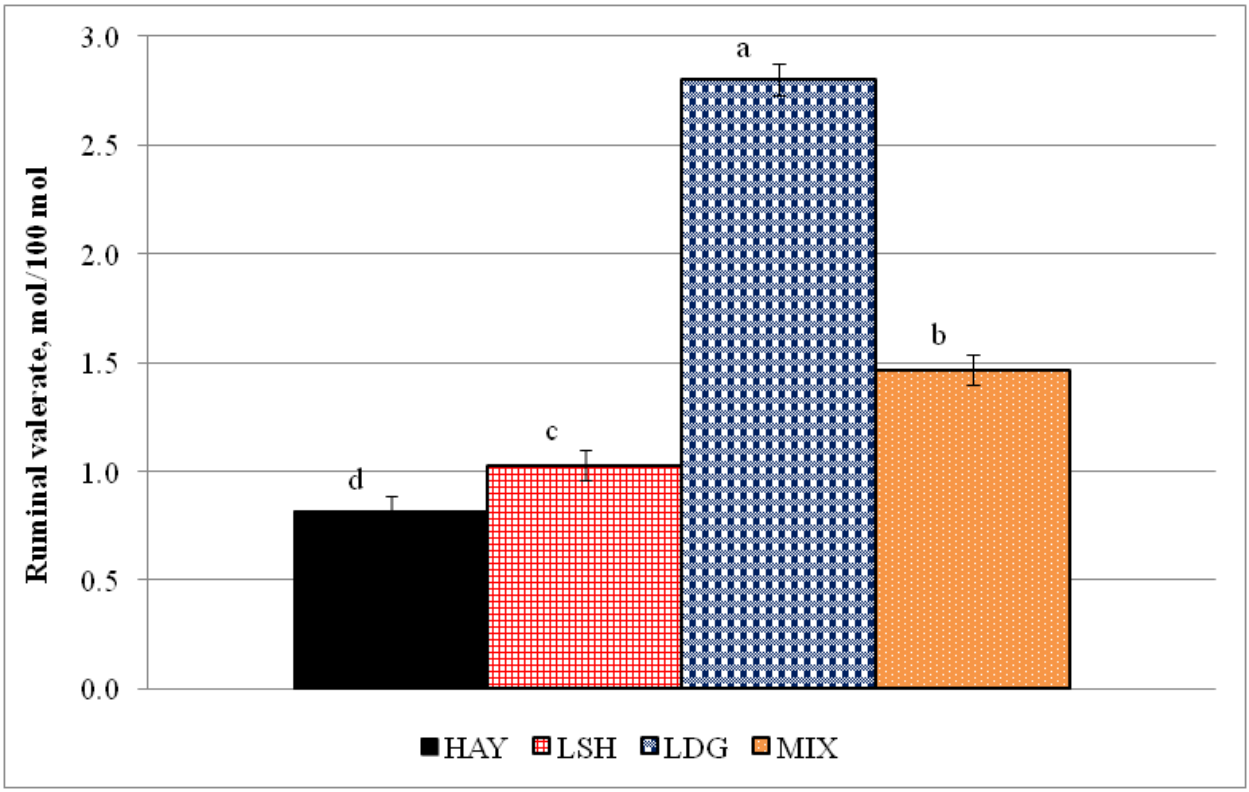

Figure 3.8. Ruminal valerate concentrations (mole/100 mole) averaged across sampling times from cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS;
Ruminal valerate concentrations varied by diet ($P < 0.01$) and time ($P < 0.01$), but not their interaction ($P = 0.34$).
[a,b,c,d]Means without a common superscript differ ($P < 0.05$).

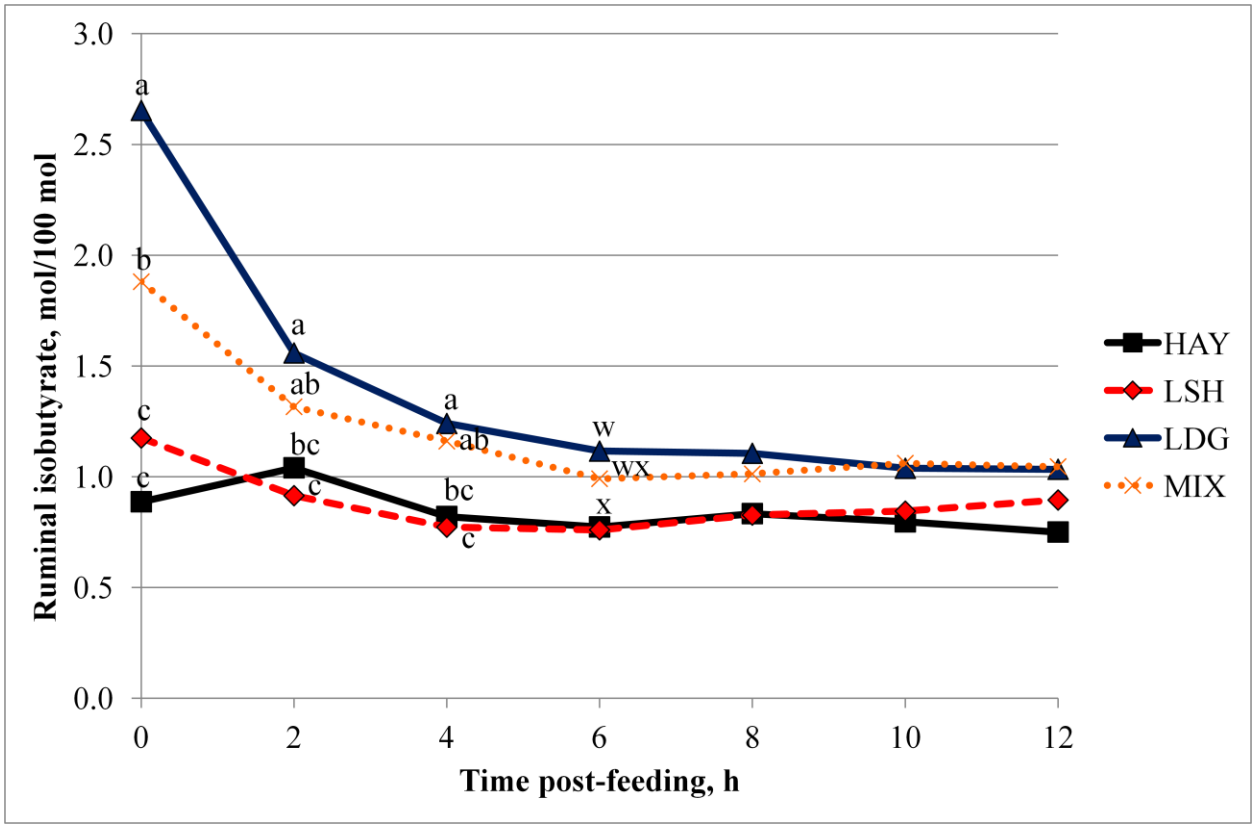

Figure 3.9. Ruminal isobutyrate concentrations (mole/100 mole) over time after feeding for cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal isobutyrate concentrations varied by diet ($P < 0.01$), time ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time without a common superscript differ ($P < 0.05$).
[w,x]Means within a time without a common superscript tend to differ ($0.05 \leq P \leq 0.10$).

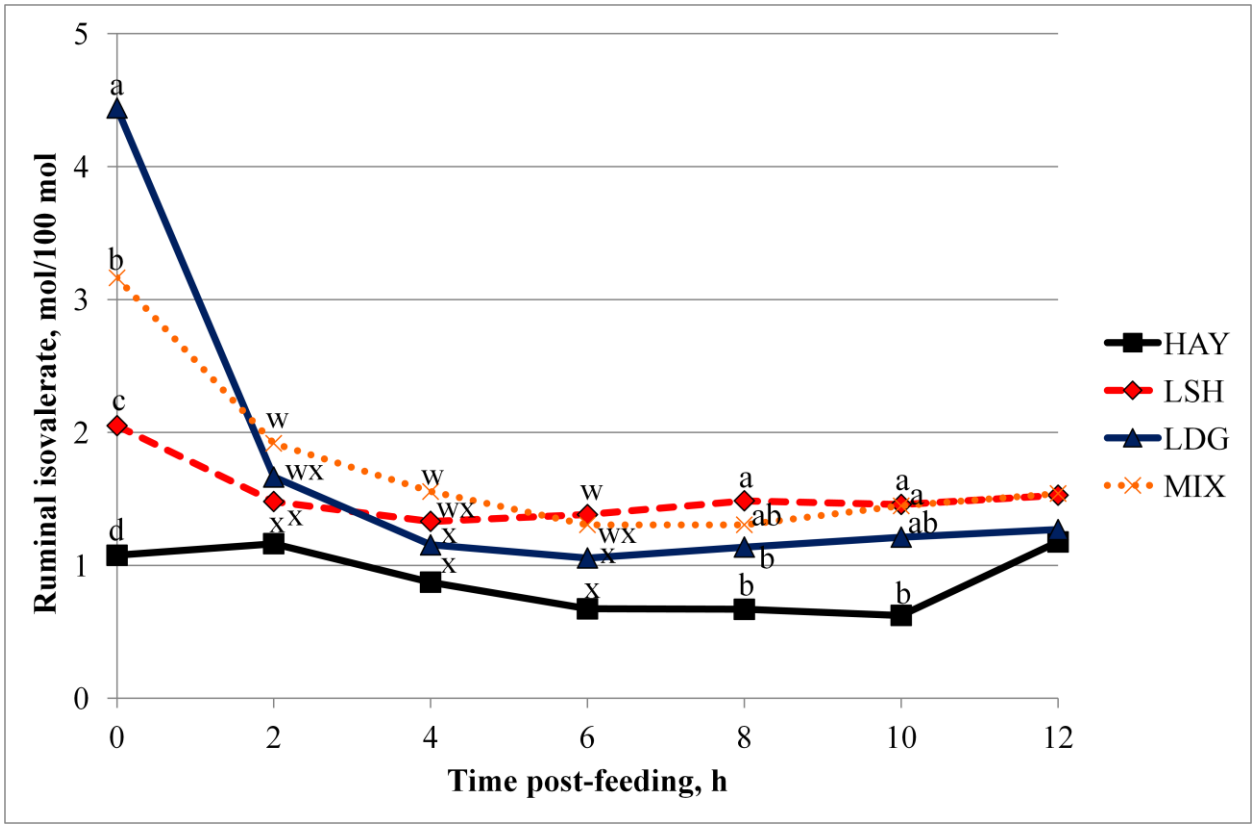

Figure 3.10. Ruminal isovalerate concentrations (mole/100 mole) over time after feeding for cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal isovalerate concentrations varied by diet ($P < 0.01$), time ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c,d]Means within a time without a common superscript differ ($P < 0.05$).
[w,x]Means within a time without a common superscript tend to differ ($0.05 \leq P \leq 0.10$).

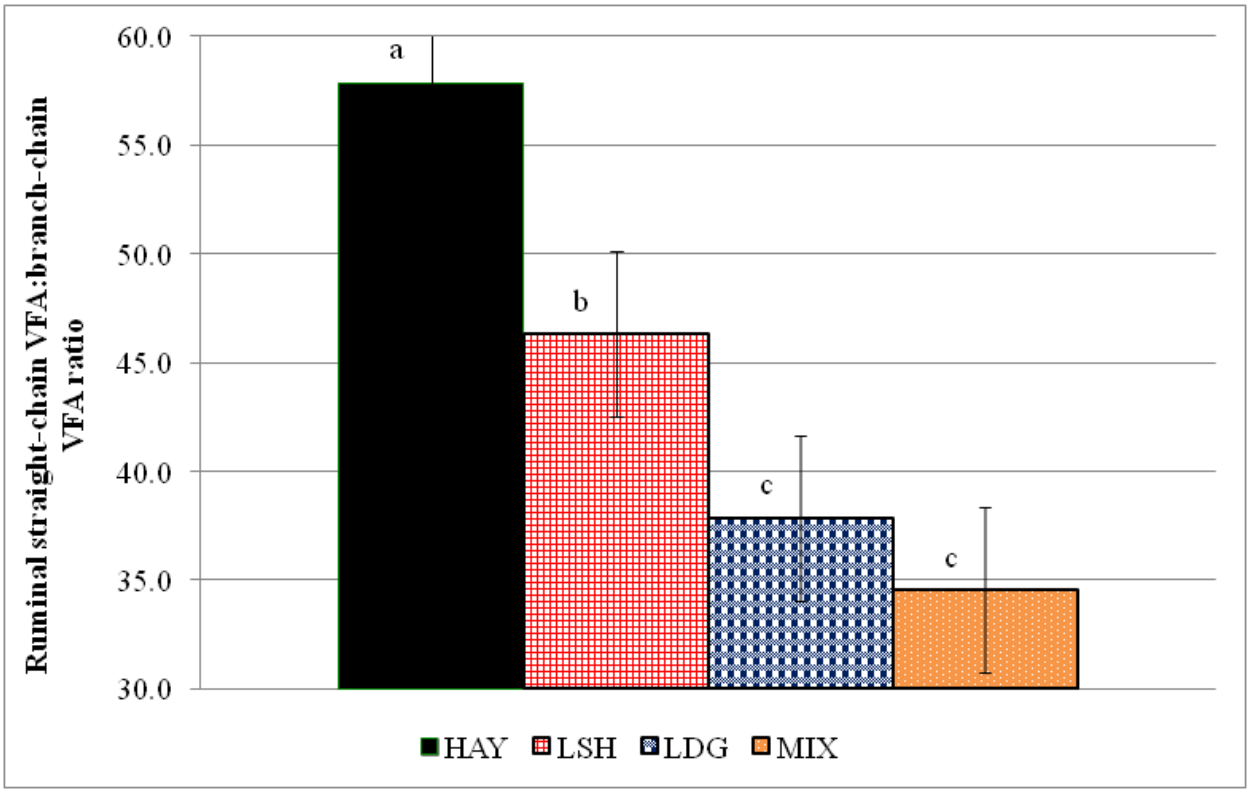

Figure 3.11. Ruminal straight-chain VFA (acetate, propionate, butyrate, valerate) to branch-chain VFA (isobutyrate, isovalerate) ratio averaged across sampling times from cows limit-fed co-product feedstuffs.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
Ruminal straight-chain VFA:branch-chain VFA ratio varied by diet ($P < 0.01$) and time ($P < 0.01$), but not their interaction ($P = 0.14$).
[a,b,c]Means without a common superscript differ ($P < 0.05$).

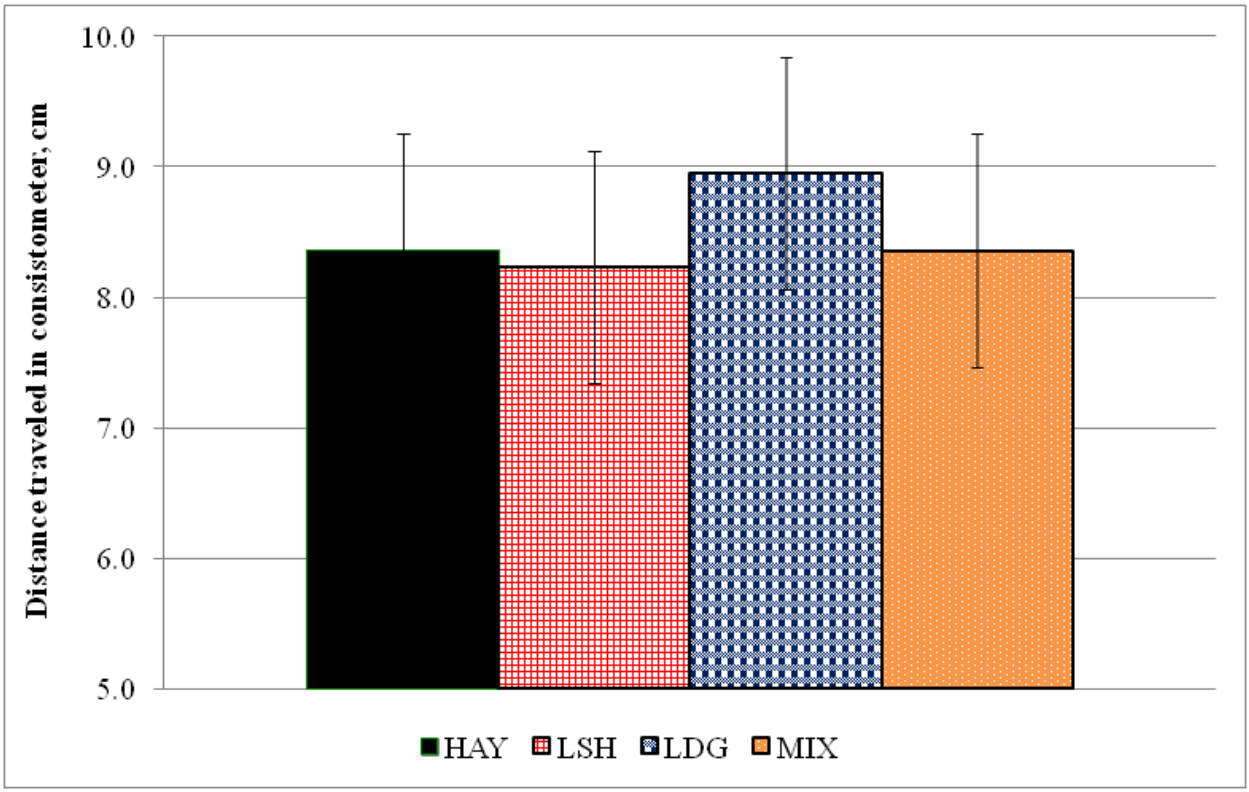

Figure 3.12. Ruminal fluid viscocity from cows limit-fed co-product feedstuffs as measured by distance travelled in a consistometer (Akins et al., 2009).
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
There was a significant effect of time (P = 0.01), but not diet (P = 0.27) or their interaction (P = 0.49).

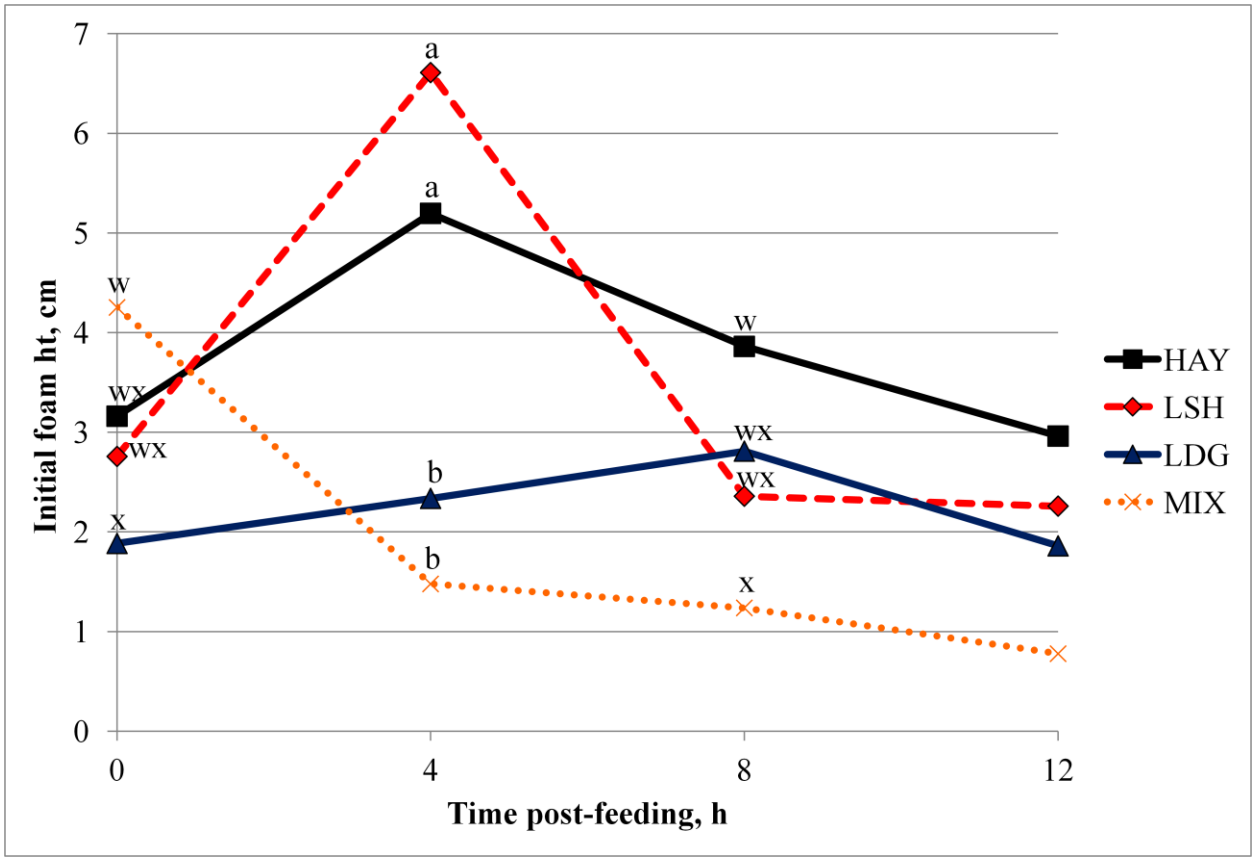

Figure 3.13. Plot of initial foam height (after aeration with CO_2 at 6.9kPa for 30 s) of ruminal fluid (50 mL) against time after feeding for cows limit-fed co-product feedstuffs (Akins et al., 2009).
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
There was a significant effect of diet ($P = 0.05$), time ($P = 0.04$), and their interaction ($P = 0.05$).
[a,b,c]Means within a time without a common superscript differ ($P < 0.05$).
[w,x]Means within a time without a common superscript tend to differ ($0.05 \leq P \leq 0.10$).

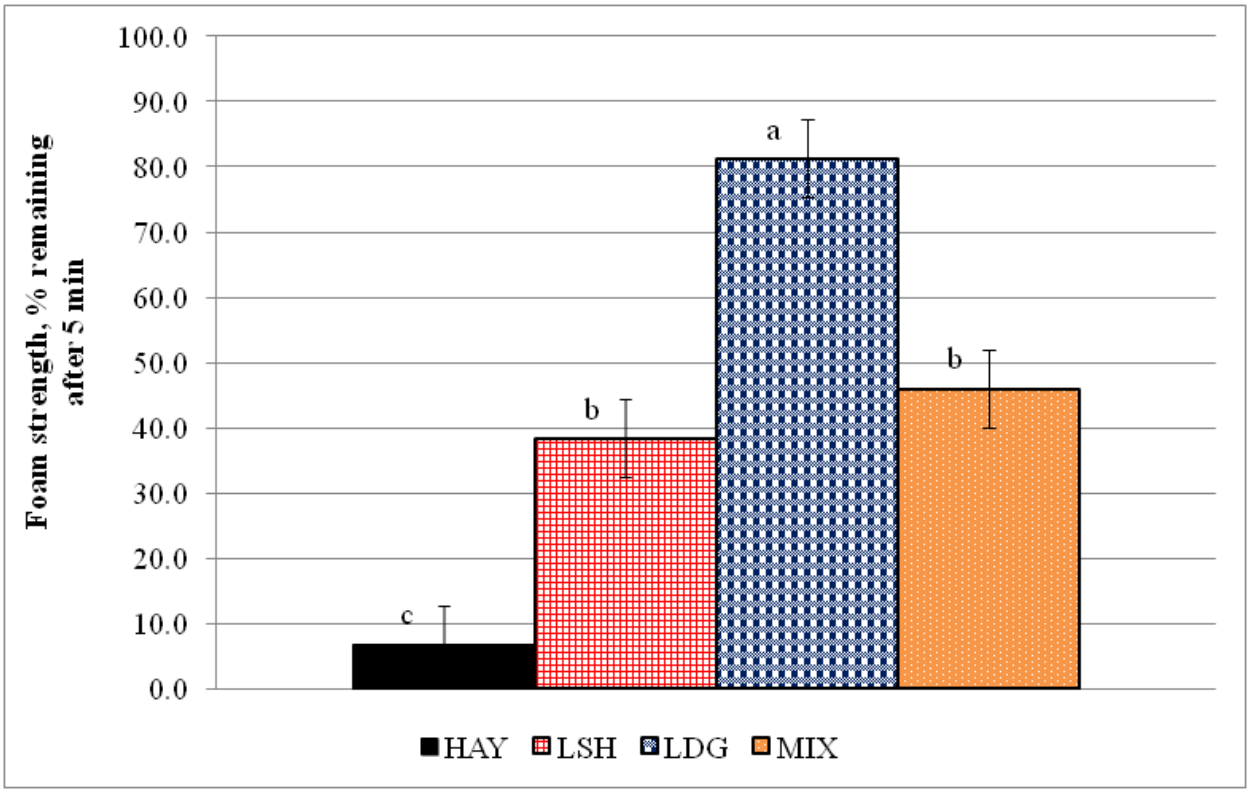

Figure 3.14. Ruminal fluid foam strength (portion of foam remaining after 5 minutes) from cows limit-fed co-product feedstuffs as measured by aeration with CO_2 (Akins et al., 2009).
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed DDGS; MIX = limit-fed a mixture of soybean hulls and DDGS.
There was a significant effect of diet($P < 0.01$), but not time ($P = 0.42$) or their interaction ($P = 0.72$).
[a,b,c]Means within a time without a common superscript differ ($P < 0.05$).

UNIVERSITY OF
ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Kenneth Coffey

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: November 20, 2012

SUBJECT: IACUC Modification Request **APPROVAL**
Expiration date : **February 6, 2015**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** the modification request (to refine experimental design) to Protocol **#12023- "Intake, digestibility, and ruminal fermentation of warm- and cool-season forages by lactating beef cows**". You may implement this Modification immediately.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any additional changes in the protocol during the research, please notify the IACUC in writing [via the Modification Request form] **prior** to initiating the changes.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

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DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

UNIVERSITY OF ARKANSAS
DALE BUMPERS COLLEGE
OF AGRICULTURAL, FOOD & LIFE SCIENCES

April 1, 2014

To Whom It May Concern:

I am writing this letter to certify that William Brandon Smith conducted the work and wrote in excess of 51% of chapter 3 of his thesis entitled "*Effect of Limit-Fed Co-product Feedstuffs on Production, Digestion, Fermentation and Rumen Function in Beef Cattle*".

Please contact me if you have further questions or concerns.

Sincerely,

Kenneth P. Coffey, Ph.D.; PAS
Professor, Beef Cattle Nutrition and Management.

ANIMAL SCIENCE DEPARTMENT
AFLS B114
University of Arkansas
Fayetteville, Arkansas 72701
479-575-4351 / Fax 479-575-7294

CHAPTER 4: RUMINAL FORAGE DIGESTIBILITY FOLLOWING A PERIOD OF LIMIT-FEEDING CO-PRODUCT FEEDSTUFFS[1]

W.B. Smith[2], K.P. Coffey[2], R.T. Rhein[2], E.B. Kegley[2], D. Philipp[2], J.D. Caldwell[3] and A.N. Young[2]

Abstract

Co-product feedstuffs may represent an economical alternative to hay as basal diet, but concentrate feedstuffs are known to affect forage utilization negatively when offered at higher levels. Our objective was to determine the time necessary for full rumen recovery of forage digestibility following a period of limit-feeding of co-product feedstuffs as the major component of the diet. Eight ruminally-fistulated cows (671 ± 32.0 kg BW) were stratified by BW and allocated randomly to 1 of 4 diets in a 2-period study: 1) limit-fed soybean hulls (**LSH**), 2) limit-fed distillers' dried grains with solubles (**LDG**), 3) limit-fed an isoenergetic mixture of the two (**MIX**), or 4) ad libitum mixed-grass hay (**HAY**). On d 5 prior to,and d 0, 7, 14, 21 and 28 following removal from diets, each of 8 test forages (bermudagrass, crabgrass, tall fescue, oat, orchardgrass, rescuegrass, HAY and tall fescue hay) were inserted in triplicate Dacron bags into the rumen of each cow for a 48-h incubation period. In situ DM disappearance was plotted against d from diet removal, and a Gompertz 3-parameter curve was fitted for each of LSH and LDG by cow using JMP® Statistical Discovery Software. An inverse prediction function was tested within each forage against the mean disappearance the same forage from cows offered

[1]This chapter has been submitted in part, to Animal Production Science as a proceedings paper for the 2014 Joint International Symposium on the Nutrition of Herbivores and the International Symposium on Ruminant Physiology.

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HAY to predict d to recovery. Asymptotes, growth rates and d to recovery were analyzed using SAS PROC MIXED. Recovery time tended to be less ($P = 0.08$) from LSH compared with LDG for bermudagrass, and recovery rate tended to be greater ($P = 0.06$) from LDG compared with LSH for orchardgrass, but parameters did not differ ($P \geq 0.14$) between diets for other forages. Therefore, cows may be limit-fed co-product feedstuffs as a majority feed source without significant short or long-term negative impacts on subsequent forage digestibility.

1. Introduction

Limit-feeding is an effective strategy for maintaining cows when hay supplies are limited (Loerch, 1996; Gunter et al., 2000). With limit-feeding, cattle are generally more efficient at maintaining (Loerch, 1996; Schoonmaker et al., 2003) or gaining (Gunter et al., 2000) BCS irrespective of fluctuations in wt, even when diets are offered to meet maintenance requirements. Most of this improvement in efficiency is attributed to improvements in diet digestibility (Galyean et al., 1979; Driedger and Loerch, 1999), which can generally be attributed to the increased digestibility of the feedstuffs provided in these systems. Offering such highly digestible feedstuffs reduced fiber digestibility in ad libitum-feeding situations (Wedekind et al., 1986; Reed et al., 1997; Stensig and Robinson, 1997). However, digestibility of both aNDF (Hoffman et al., 2007; Felix et al., 2011) and ADF (Murphy and Loerch, 1994) were improved in certain limit-feeding situations, possibly because of a reduction in aNDF intake (Hoffman et al., 2007), reduced passage rate (Murphy and Loerch, 1994),or increased hindgut fermentation (Lewis and Dehority, 1985). Limited studies reported no long-term impacts on performance following programs where high-concentrate diets were limit-fed to cows (Loerch, 1996; Gunter et al., 2000) but the time required for the rumen to adapt back to an all, or mostly, roughage diet has not been reported. Therefore, our objectives were to determine the degree to which limit-fed

co-product feedstuffs decreased ruminal forage digestibility and to determine the time necessary for full rumen recovery to steady-state digestive function.

2. Materials and Methods

2.1 Animals, forages and design

Eight ruminally-fistulated cows (672 ± 32.0 kg initial BW; approximately 9 yr of age) were used in a 2-period study to evaluate 4 different diets. In each period, cows were stratified by BW and allocated randomly to 1 of 4 diets (2 cows/diet) in a generalized complete block design: 1) limit-fed soybean hulls (**LSH**), 2) limit-fed distillers' dried grains with solubles (**LDG**), 3) limit-fed an isoenergetic mixture of SH and DDGS (**MIX**), or 4) provided ad libitum access to hay (**HAY**). Diets were formulated to meet ME requirements of an 11-month post-partum mature beef cow based on the published nutritional composition of each feedstuff and ground limestone was added to the LDG and MIX diets to equalize dietary Ca concentrations (NRC, 2000). Cows receiving limit-fed diets were offered 0.9 kg hay daily for roughage consumption. Cows on HAY were offered 0.9 kg of an isoenergetic mixture of soybean hulls and distillers' dried grains with solubles (**SHDG**) to ensure a non-limiting rumen environment

Eight different forages were used to evaluate ruminal recovery time following limit-feeding of the co-product diets. Six forages were harvested from the Watershed Research and Education Center at the University of Arkansas, Fayetteville, AR, USA, in October 2012. Forages collected included bermudagrass (**BER**; *Cynodon dactylon* [L.] Pers.), crabgrass (**CRB**; *Digitaria ciliaris* [Retz.] Koeler), tall fescue (**FES**; *Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.), oat (**OAT**; *Avena sativa* L.), orchardgrass (**ORC**; *Dactylis glomerata* L.), and rescuegrass (**RES**; *Bromus catharticus* Vahl). Additionally, tall fescue hay (**TFH**) was collected for use from Lincoln University (Jefferson City, MO, USA), and samples of HAY

actually offered to the cows as part of their diet was used as a control. Upon collection, forages were immediately frozen at -20°C until further processing. Forages were dried to a constant weight at 50°C in a forced-air oven, and ground to pass through a 2-mm screen using a Wiley mill (Arthur H. Thomas, Philadelphia, PA, USA).

Following each period, cows were housed on a dormant orchardgrass pasture with ad libitum access to HAY plus 0.9 kg of SHDG. On d 5 prior to, and d 0, 7, 14, 21 and 28 following removal from diets in each period, 24 Dacron bags, each containing 3 g of 1 of the 8 test forages were inserted in triplicate into the rumen of each cow for a 48-h incubation to determine DM disappearance (**ISD**). In situ procedures were carried out according to the procedures of Vanzant et al. (1998).

2.2 Chemical analyses and analytical procedures

Organic matter was determined on all forages via combustion in a muffle furnace (Method 942.05; AOAC, 2000). Neutral-detergent fiber and ADF were measured sequentially using the ANKOM$^{200/220}$ Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA; Vogel et al., 1999). Acid detergent lignin was determined using the sulfuric acid method (Method 973.18; AOAC, 2000). All values for laboratory analyses were corrected to a DM basis (Method 934.01; AOAC, 2000).

2.3 Statistical analyses

Time-series data (ISD across time) were analyzed within forage using the mixed models procedure of SAS® (SAS Institute, Cary, NC, USA). The model included the fixed effects of diet, interval removed from diet and their interaction, and interval was then used as a repeated measure with cow as the subject. The random statement included effects of cow and period.

If a depression in ISD was determined for a diet relative to HAY while the cows were still consuming their particular diets (d -5), time to recovery was determined by regressing ISD against interval from diet removal using JMP® Statistical Discovery Software (SAS Institute, Cary, NC, USA).A Gompertz 3-parameter sigmoidal curve was fitted for diet by cow, and an inverse prediction function was used to assess diets reaching a baseline value (the mean ISD for the particular forage from cows offered HAY) to predict d to recovery. Recovery rates and d to recovery were then analyzed using the mixed models procedure of SAS. The model included the fixed effect of diet. The random statement included effect of period. Means were reported as least squares means, and diets were separated using pair-wise *F*-protected *t*-tests. Statistical significance was declared when $P < 0.05$, and a tendency for significance was quantified when $0.05 \leq P \leq 0.10$.

3. Results

Chemical composition of forages used for in situ disappearance measurements are presented in Table 4.1. With respect to forages harvested in October, warm-season forages generally had greater concentrations of aNDF than did cool-season forages. This is likely due to the time of yr in which these samples were collected. Warm-season grasses were in the reproductive stages of growth while cool-season forages were in a vegetative growth stage. Concentrations of ADF were greater in warm-season forages, but concentrations of ADL were not as clearly distinguishable into warm- and cool-season categories.

In situ DM disappearance data for each forage is presented by cow diet and interval from diet removal in Figures 4.1 through 4.8. Across all forages, ISD was not different ($P \geq 0.12$) from cows offered MIX compared with those offered HAY while consuming the treatment diet (d -5). However, initial (d -5) ISD of all forages was lower ($P < 0.05$) from cows receiving LSH

and LDG compared with those from cows offered HAY and MIX and that by cows offered LDG was lower ($P < 0.05$) compared with cows offered LDG. The ISD was reduced by between 2.6 and 14.9% for LSH and between 4.3 and 40.6% for LDG at d -5.

On the d of removal from the diets (d 0), with incubation ending 2 d post-removal, there was no difference ($P \geq 0.17$) in ISD from those cows receiving HAY, MIX or LSH for BER, FES, TFH or HAY, but was lower ($P \leq 0.02$) from those cows receiving LDG. For CRB, ORC and RES, ISD from cows receiving LSH was intermediate ($P \leq 0.03$) to those from HAY and MIX and those from LDG. For OAT, ISD was greatest ($P < 0.05$) from cows receiving MIX, intermediate from those receiving HAY or LSH, and least from those receiving LDG. With the exception of TFH and OAT, there was no difference ($P \geq 0.11$) among diets for ISD at 7, 14, 21 or 28 d after removal from treatment diets. For TFH, ISD from cows receiving LSH tended to be intermediate ($P = 0.05$) to those from HAY and MIX and those from LDG. There was an unexplained anomaly for d 21 from OAT. At this time, ISD was greatest ($P = 0.03$) from cows offered LDG, intermediate from LSH and MIX, and least from HAY, though values were only over a range of 15 g/kg.

Since ISD from LSH and LDG differed ($P < 0.05$) from HAY while diets were offered (d -5), but MIX did not, only LSH and LDG were tested for time to rumen recovery. Asymptotes of the nonlinear regression model, which would signify maximal ISD, did not differ ($P \geq 0.24$) between LSH and LDG for any forages but BER, for which the asymptote from LSH tended to be lower ($P = 0.10$) compared with LDG (Table 4.2). Rate of recovery tended to be slower ($P = 0.06$) from LSH compared with LDG for ORC, but did not differ ($P \geq 0.14$) for other forages. There tended to be a shorter recovery time ($P = 0.08$) from LSH compared with LDG for BER, but statistical significance was not achieved for any other forage ($P \geq 0.22$).

When grouping the test forages by growth type or seasonality (Table 4.3), there was no difference ($P \geq 0.79$) between diets for asymptotes or recovery rates for annual or perennial forages, or for cool- or warm-season varieties. However, cows from LDG tended to have longer ($P = 0.09$) recovery times for annual forages than from LSH, but recovery times did not differ ($P \geq 0.29$) between LSH and LDG for perennial forages, or cool- or warm-season species.

4. Discussion

The effect of concentrate addition to the diet on forage digestion in vivo has been well characterized (Miller and Muntifering, 1985). When concentrate was increased as a proportion of the diet offered to a maximum of 800 g/kg, potential and apparent extents of digestion of forage fiber were reduced to nearly half of the starting point, and fiber passage rate was significantly reduced. The same was true of starch addition in vitro, though no linear relationship could be established (Mertens and Loften, 1980). However, each of these groups examined the effects of concentrate on forage digestion when the two were fed in combination. Under a limit-feeding scenario, forage offering would be minimal and would only be resumed when the limited forage supply is replenished, presumably following the limit-feeding period.

Ruminal pH, as affected by diet offered, has been shown to have a greater effect on forage digestion than on concentrate digestion in cattle (Calsamiglia et al., 2008). A negative linear relationship of 14.9 units reduction in OM disappearance for each unit decline in pH was observed (Calsamiglia et al., 2008). In the present study, pH of the rumen environment while consuming the diet may have led to the depression in ISD observed in those animals from LSH and LDG. Others, though, observed no decrease in ruminal forage fiber digestion rates with increasing level of concentrate, but, instead, noted a decrease in passage rate (Miller and Muntifering, 1985). Since passage rate could not affect the forages tested in situ in the present

study, the conclusion may be drawn that a diet based solely on co-product feedstuffs actually affects the rate, or at least the extent, of forage DM disappearance.

Limitations of the regression method used for prediction of recovery times were uncovered in the results obtained. Negative d to recovery (such as those with RES) would indicate a recovery to baseline forage DM digestibility prior to being removed from the diet, even though a depression in DM disappearance was observed while the diet was being offered. Values for recovery in excess of 28 d are unrealistic in this dataset. Since diets did not differ beyond d 0 (incubation concluding on d 2), this seems quite unlikely. Since the rumen was in a constant state of change, and Dacron bags were allowed to incubate 48 h during this change, it is likely that minute differences in recovery time were not evident in the data collected. It is possible that in vitro assays using rumen fluid collected at various times during the recovery period would be a better tool to capture snapshots of individual moments in the dynamic recovery of the rumen environment.

5. Conclusion

In the present study, forage in situ disappearance was reduced in comparison to cows consuming a hay diet when single co-products were limit-fed, but the positive associative effect of a mixed co-product diet alleviated this depression. Of the eight forages used to evaluate ruminal recovery from limit-feeding different co-product diets, only the recovery time for bermudagrass was different between previous diets. No statistical significance was observed for recovery time when forages were grouped by seasonality (cool-season or warm-season) or for perennial forages. Therefore, co-products can be limit-fed to meet ME requirements of beef cattle without significant adverse effects on subsequent forage digestion when animals are returned to a forage-based diet.

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Table 4.1. Chemical analysis of forages used for in situ disappearance measurements to characterize the effects of previous diet on ruminal recovery time

Item[1]	BER[2]	CRB	FES	OAT	ORC	RES	HAY	TFH
DM (g/kg)	868	862	378	184	382	319	894	943
OM (g/kg DM)	876	847	876	869	884	800	880	867
NDF (g/kg DM)	704	605	518	393	570	506	669	622
ADF (g/kg DM)	317	312	251	199	297	279	355	347
ADL (g/kg DM)	48	49	24	24	53	42	56	46
HCell (g/kg DM)	387	293	267	194	274	227	314	275
Cell (g/kg DM)	265	258	223	172	241	233	291	293

[1]DM = dry matter; OM = organic matter; NDF = neutral-detergent fiber; ADF = acid-detergent fiber; ADL = acid detergent lignin; Hcell = hemicellulose; Cell = cellulose.

[2]BER = bermudagrass; CRB = crabgrass; FES = tall fescue; OAT = oat; ORC = orchardgrass; RES = rescuegrass; HAY = control hay; TFH = tall fescue hay.

Table 4.2. Inverse prediction parameters, by forage, for rumen recovery following a period of limit-feeding co-product feedstuffs to meet cow energy requirements

Item[1]	LSH[2]	LDG	SEM	*P*-value[3]
Bermudagrass				
Asymptote(g/kg ISD)	508[x]	517[w]	5.4	0.10
Recovery rate (/d)	0.3	0.2	0.04	0.22
Recovery time (d)	4.1[x]	11.4[w]	2.19	0.08
Crabgrass				
Asymptote (g/kg ISD)	657	647	15.2	0.49
Recovery rate (/d)	0.2	0.3	0.07	0.60
Recovery time (d)	7.0	9.5	6.02	0.40
Tall fescue				
Asymptote (g/kg ISD)	826	832	9.1	0.27
Recovery rate (/d)	0.6	0.3	0.26	0.33
Recovery time (d)	2.5	6.9	5.37	0.42
Oat				
Asymptote (g/kg ISD)	957	959	0.2	0.41
Recovery rate (/d)	0.6	0.4	0.31	0.35
Recovery time (d)	2.5	7.6	7.16	0.22
Orchardgrass				
Asymptote (g/kg ISD)	793	788	4.5	0.34
Recovery rate (/d)	0.1[x]	0.4[w]	0.11	0.06
Recovery time (d)	42.4	6.5	23.14	0.32
Rescuegrass				
Asymptote (g/kg ISD)	912	913	11.9	0.62
Recovery rate (/d)	0.4	0.7	0.38	0.48
Recovery time (d)	-2.0	-1.2	2.12	0.81
Control hay				
Asymptote (g/kg ISD)	677	679	11.1	0.80
Recovery rate (/d)	0.2	0.2	0.08	0.14
Recovery time (d)	11.8	12.0	9.90	0.96
Tall fescue hay				
Asymptote (g/kg ISD)	717	724	9.2	0.24
Recovery rate (/d)	0.2	0.2	0.04	1.00
Recovery time (d)	5.0	9.1	5.02	0.32

[1]ISD = 48-h in situ forage dry matter disappearance.
[2]LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles.
[3]*P*-value reported is for the main effect of diet.
[w,x]Means within a row without a common superscript letter tend to differ ($P < 0.10$).

Table 4.3. Inverse prediction parameters, by seasonality and growth type, for rumen recovery following a period of limit-feeding co-product feedstuffs to meet cow energy requirements

Item[1]	LSH[2]	LDG	SEM	*P*-value[3]
---------- Growth Type ----------				
Annuals[4]				
Asymptote (g/kg ISD)	842	840	40.9	0.97
Recovery rate (/d)	0.4	0.4	0.21	0.81
Recovery time (d)	4.6[x]	7.9[w]	3.25	0.09
Perennials[5]				
Asymptote (g/kg ISD)	705	708	26.0	0.93
Recovery rate (/d)	0.3	0.3	0.09	0.79
Recovery time (d)	15.7	9.1	5.83	0.43
---------- Seasonality ----------				
Cool season[6]				
Asymptote (g/kg ISD)	841	843	19.6	0.92
Recovery rate (/d)	0.4	0.4	0.19	0.94
Recovery time (d)	12.3	6.3	6.26	0.51
Warm season[7]				
Asymptote (g/kg ISD)	607	614	22.8	0.83
Recovery rate (/d)	0.2	0.2	0.03	0.87
Recovery time (d)	7.6	11.0	4.97	0.29

[1]ISD = 48-h in situ forage dry matter disappearance.
[2]LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles.
[3]*P*-value reported is for the main effect of diet.
[4]Crabgrass and oat.
[5]Bermudagrass, tall fescue, orchardgrass, rescuegrass, control hay and tall fescue hay.
[6]Tall fescue, oat, orchardgrass, rescuegrass and tall fescue hay.
[7]Bermudagrass, crabgrass and control hay.
[w,x]Means within a row without a common superscript letter tend to differ ($P < 0.10$).

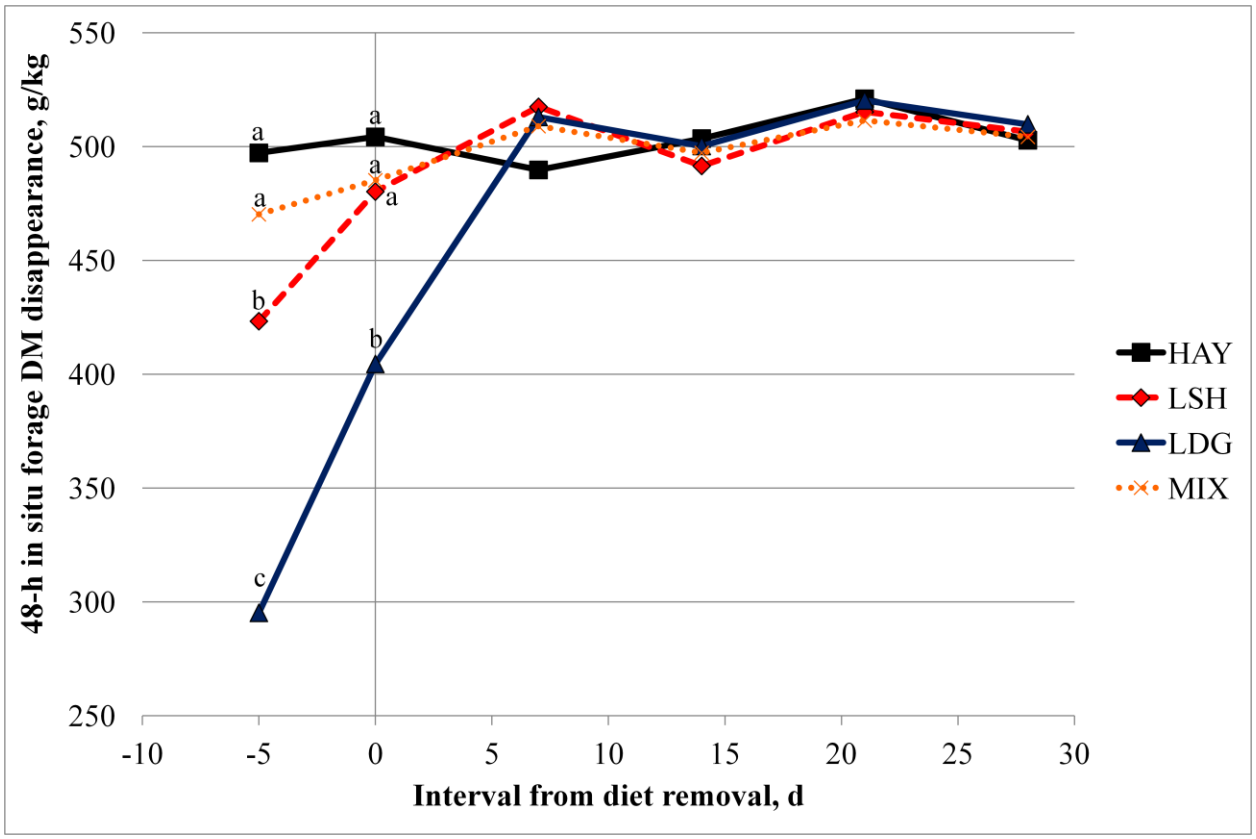

Figure 4.1. Plot of 48-h in situ bermudagrass (BER; *Cynodon dactylon* [L.] Pers.) DM disappearance versus d removed from treatment diets.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed a mixture of soybean hulls and distillers' dried grains with solubles.
There was a significant effect of diet ($P < 0.01$), interval ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time (interval) without a common superscript differ ($P < 0.05$).

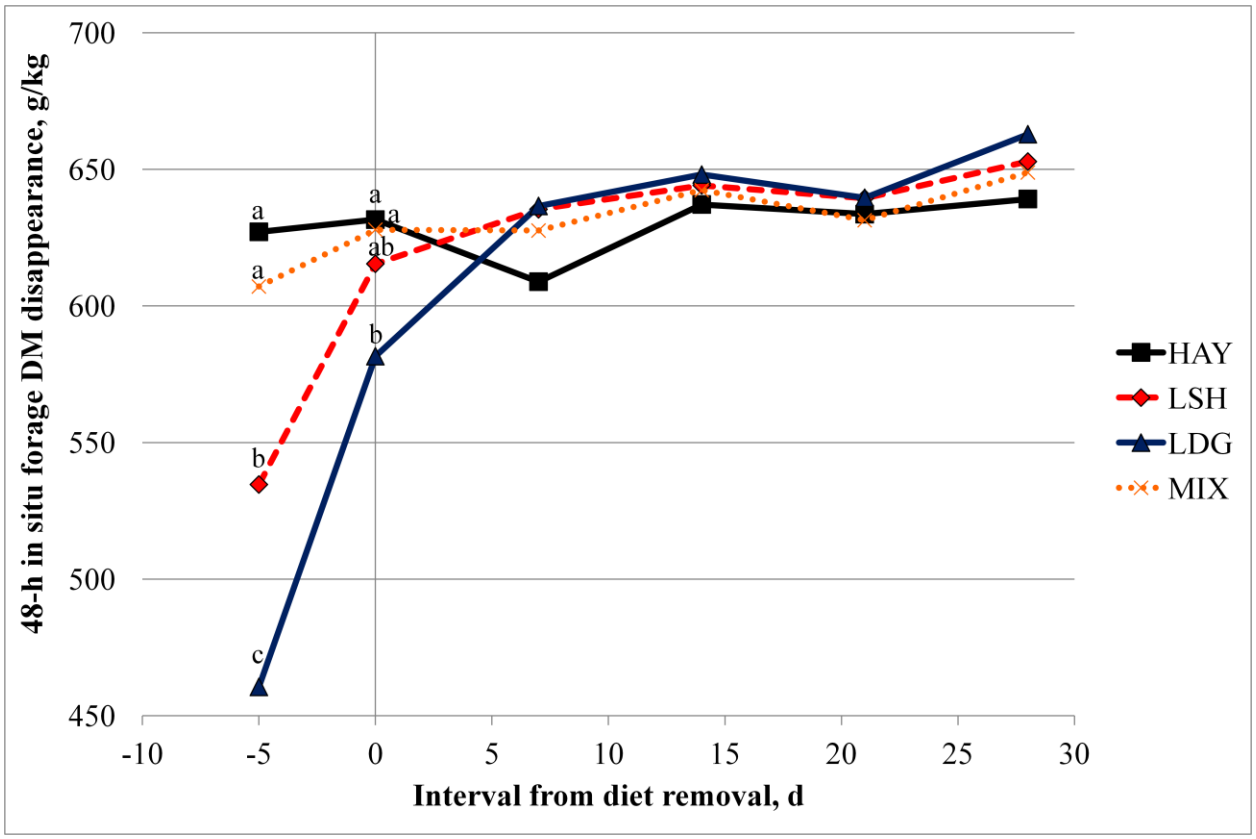

Figure 4.2. Plot of 48-h in situ crabgrass (CRB; *Digitaria ciliaris* [Retz.] Koeler) DM disappearance versus d removed from treatment diets.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed a mixture of soybean hulls and distillers' dried grains with solubles.
There was a significant effect of diet ($P < 0.01$), interval ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time (interval) without a common superscript differ ($P < 0.05$).

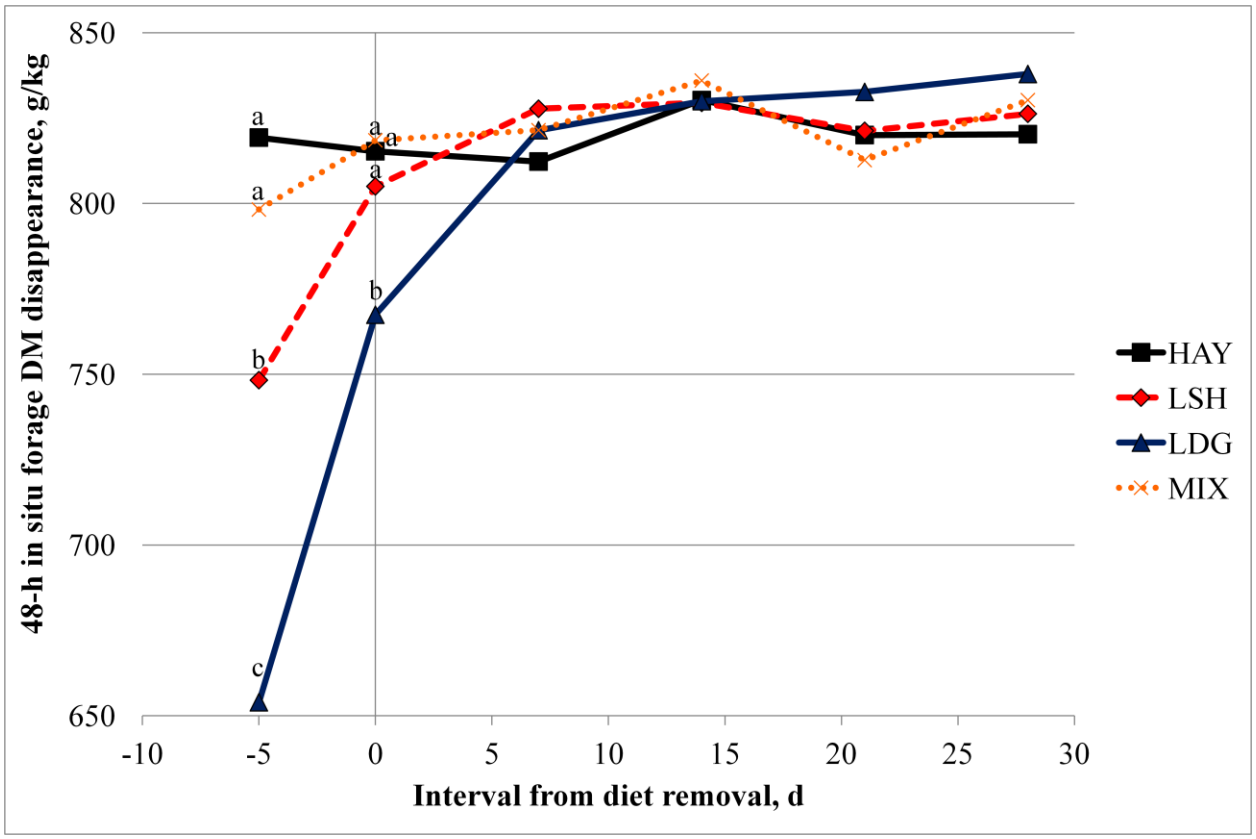

Figure 4.3. Plot of 48-h in situ tall fescue (FES; *Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) DM disappearance versus d removed from treatment diets.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed a mixture of soybean hulls and distillers' dried grains with solubles.
There was a significant effect of diet ($P < 0.01$), interval ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time (interval) without a common superscript differ ($P < 0.05$).

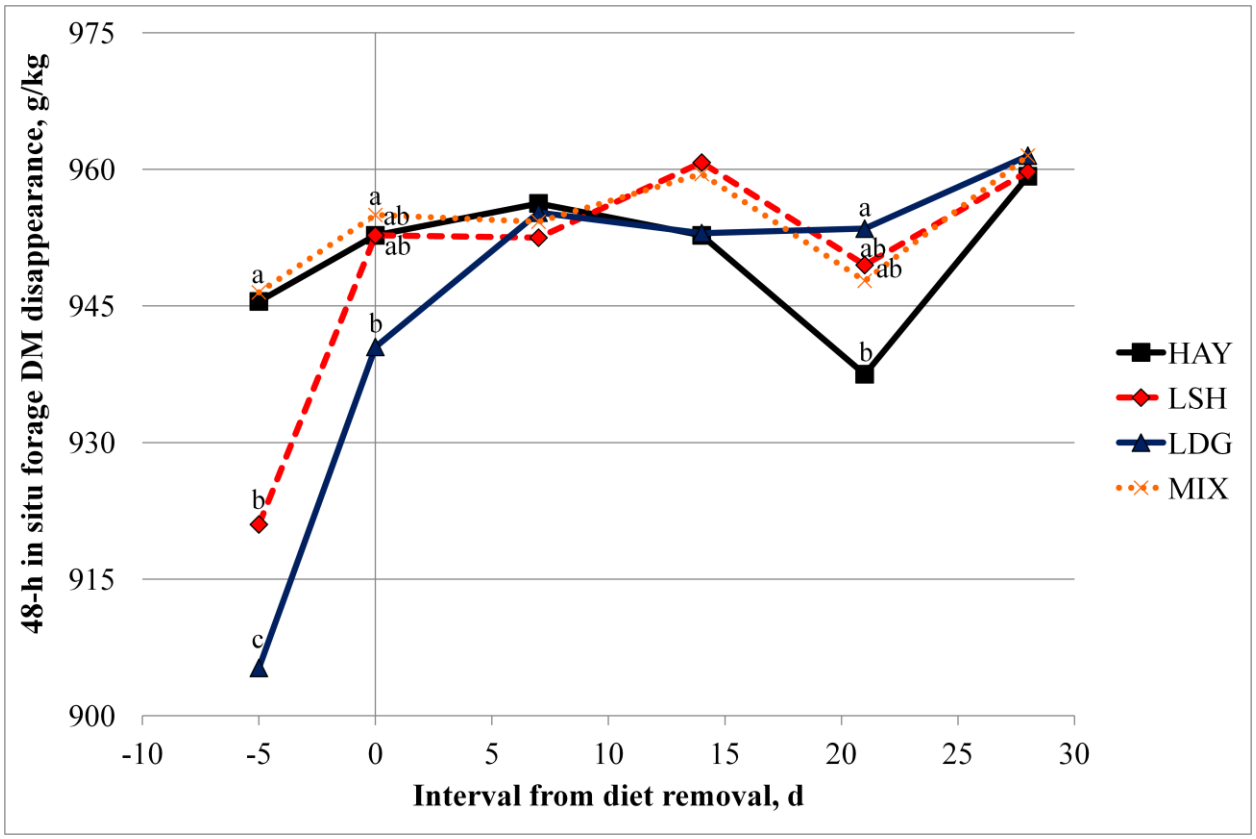

Figure 4.4. Plot of 48-h in situ oat (OAT; *Avena sativa* L.) DM disappearance versus d removed from treatment diets.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed a mixture of soybean hulls and distillers' dried grains with solubles.
There was a significant effect of diet ($P = 0.02$), interval ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time (interval) without a common superscript differ ($P < 0.05$).

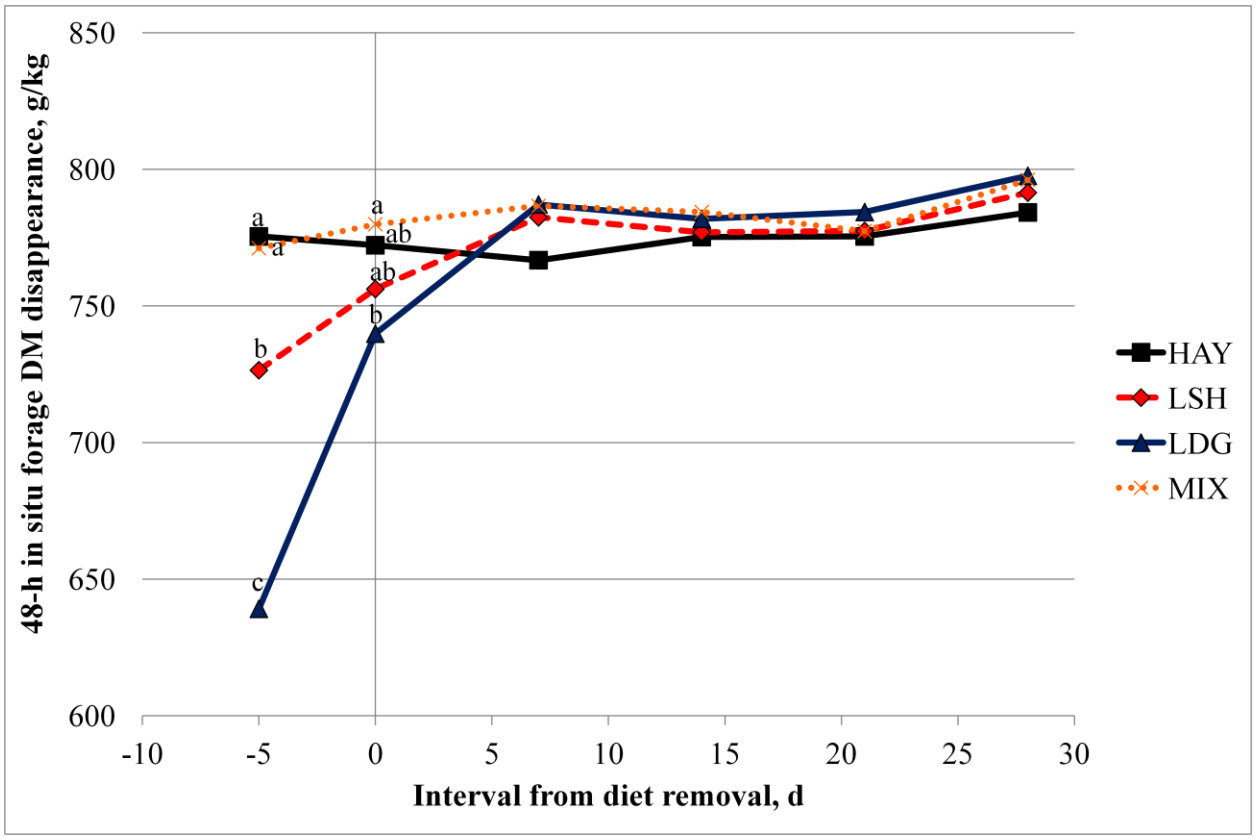

Figure 4.5. Plot of 48-h in situ orchardgrass (ORC; *Dactylis glomerata* L.) DM disappearance versus d removed from treatment diets.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed a mixture of soybean hulls and distillers' dried grains with solubles.
There was a significant effect of diet ($P < 0.01$), interval ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time (interval) without a common superscript differ ($P < 0.05$).

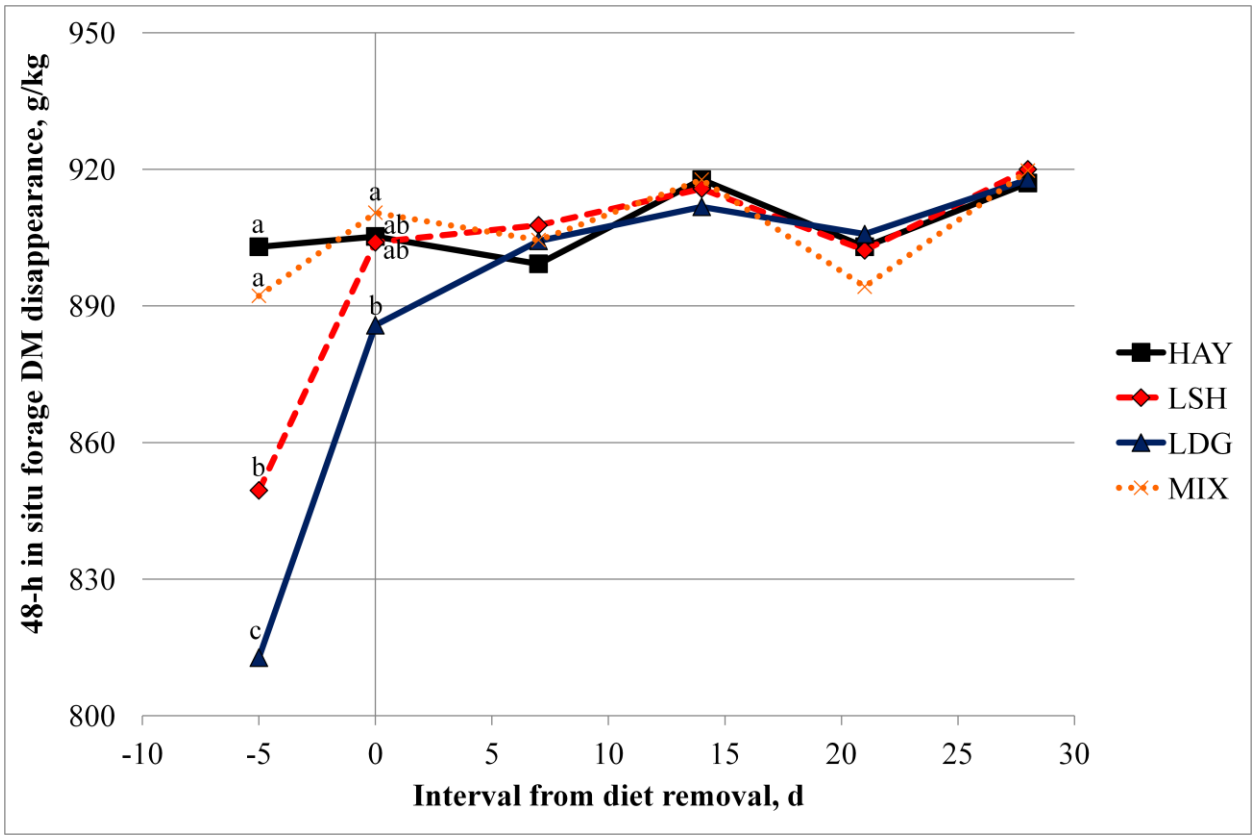

Figure 4.6. Plot of 48-h in situ rescuegrass (RES; *Bromus catharticus* Vahl) DM disappearance versus d removed from treatment diets.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed a mixture of soybean hulls and distillers' dried grains with solubles.
There was a significant effect of diet ($P < 0.01$), interval ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time (interval) without a common superscript differ ($P < 0.05$).

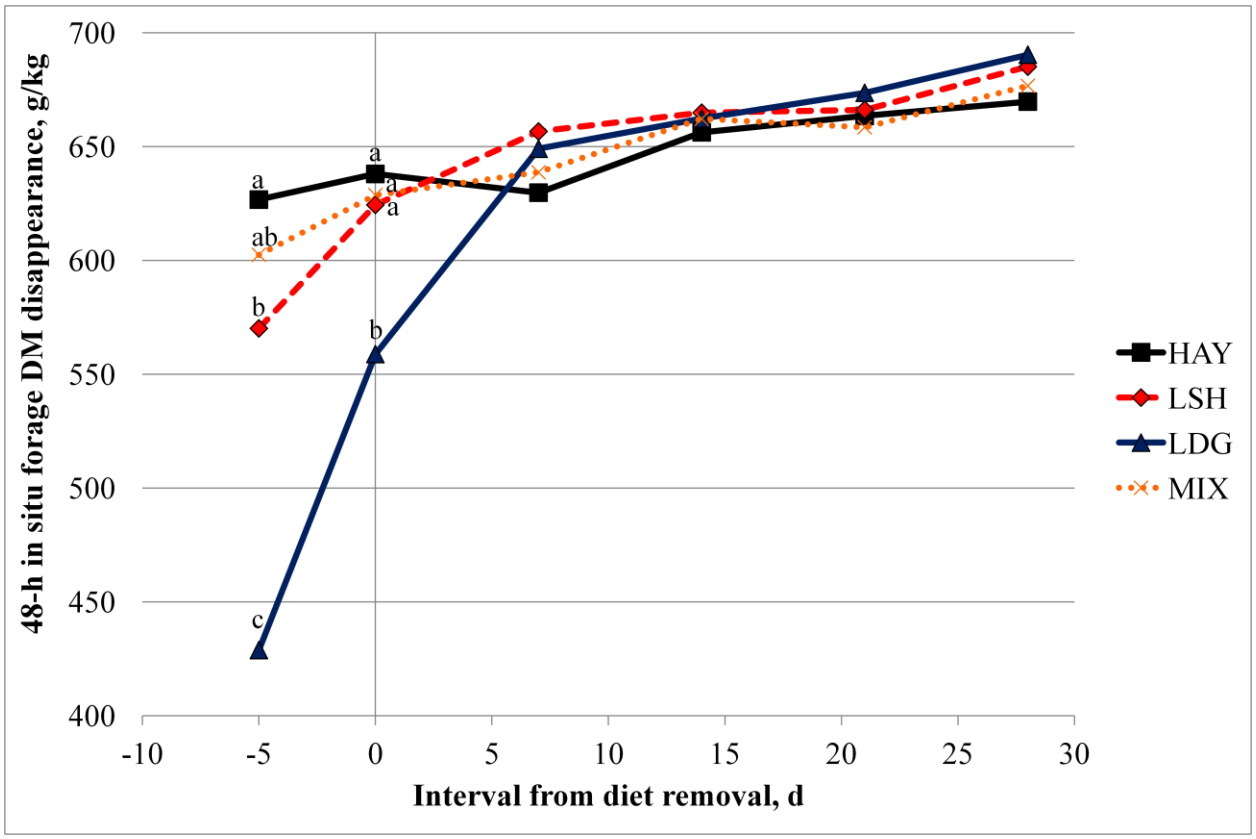

Figure 4.7. Plot of 48-h in situ control hay (HAY) DM disappearance versus d removed from treatment diets.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed a mixture of soybean hulls and distillers' dried grains with solubles.
There was a significant effect of diet ($P < 0.01$), interval ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time (interval) without a common superscript differ ($P < 0.05$).

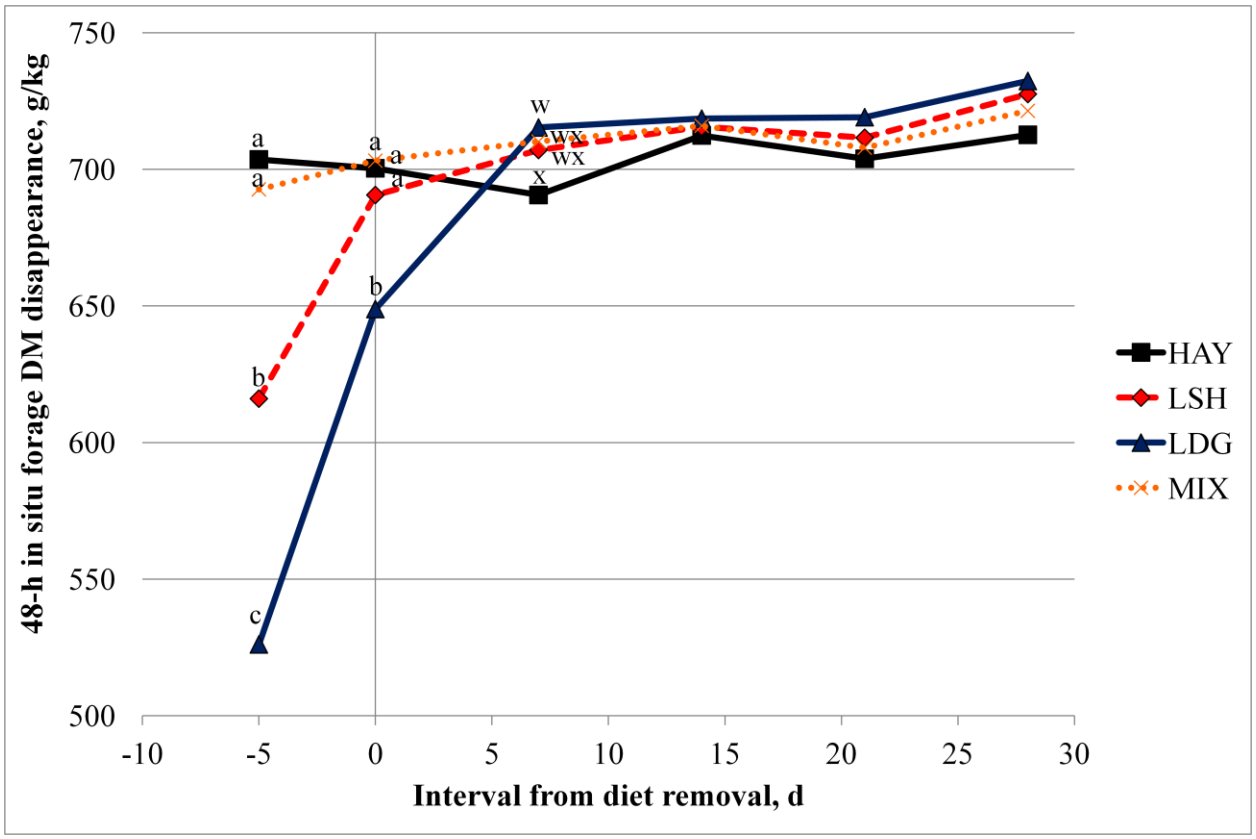

Figure 4.8. Plot of 48-h in situ tall fescue hay (TFH; *Schedonorus arundinaceus* [Schreb.] Dumort., nom. cons.) DM disappearance versus d removed from treatment diets.
HAY = ad libitum hay; LSH = limit-fed soybean hulls; LDG = limit-fed distillers' dried grains with solubles; MIX = limit-fed a mixture of soybean hulls and distillers' dried grains with solubles.
There was a significant effect of diet ($P < 0.01$), interval ($P < 0.01$), and their interaction ($P < 0.01$).
[a,b,c]Means within a time (interval) without a common superscript differ ($P < 0.05$).
[w,x]Means within a time (interval) without a common superscript tend to differ ($P < 0.10$).

UNIVERSITY OF
ARKANSAS

Office of Research Compliance

MEMORANDUM

TO: Kenneth Coffey

FROM: Craig N. Coon, Chairman
Institutional Animal Care
And Use Committee

DATE: November 20, 2012

SUBJECT: IACUC Modification Request **APPROVAL**
Expiration date : **February 6, 2015**

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** the modification request (to refine experimental design) to Protocol **#12023- "Intake, digestibility, and ruminal fermentation of warm- and cool-season forages by lactating beef cows**". You may implement this Modification immediately.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any additional changes in the protocol during the research, please notify the IACUC in writing [via the Modification Request form] **prior** to initiating the changes.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

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DIVISION OF AGRICULTURE
RESEARCH & EXTENSION
University of Arkansas System

UNIVERSITY OF ARKANSAS
DALE BUMPERS COLLEGE
OF AGRICULTURAL, FOOD & LIFE SCIENCES

April 1, 2014

To Whom It May Concern:

I am writing this letter to certify that William Brandon Smith conducted the work and wrote in excess of 51% of chapter 4 of his thesis entitled "*Effect of Limit-Fed Co-product Feedstuffs on Production, Digestion, Fermentation and Rumen Function in Beef Cattle*".

Please contact me if you have further questions or concerns.

Sincerely,

Kenneth P. Coffey, Ph.D.; PAS
Professor, Beef Cattle Nutrition and Management.

ANIMAL SCIENCE DEPARTMENT
AFLS B114
University of Arkansas
Fayetteville, Arkansas 72701
479-575-4351 / Fax 479-575-7294

CHAPTER 5: CONCLUSION

The overall goal of the set of experiments presented in this thesis was to characterize the effects of co-product feedstuffs, specifically soybean hulls (SH) and distillers' dried grains with solubles (DDGS), on cow and calf performance, cow digestive and fermentative function, and subsequent ruminal fiber digestion following removal from the diet. In chapter 2, performance by cows limit-fed soybean hulls (LSH) was similar to cows allowed ad libitum access to bermudagrass hay (HAY) in all parameters measured (BW, BCS and serum NEFA, as well as calf growth-performance measurements). Limit-feeding, though, may not be appropriate for heifers, who appeared to mobilize fat to a greater extent than primiparous or multiparous cows when limit-fed soybean hulls. Overall, LSH represented a savings of almost $22 per cow over the course of the 68-d study.

Results from chapter 3 seem to support the observations of chapter 2. Contrary to the design, DM and OM intake did not differ between HAY and the mean of the limit-fed diets, but digestibility of all dietary components was improved with limit-feeding, and apparent absorption of N also tended to be improved with limit-feeding. Limit-feeding co-product feeds did lower ruminal pH, but this was not to an extent as to inhibit adequate digestive function. The increase in total VFA from the cows limit-fed soybean hulls further supports the adequacy of LSH for gestating cows. Inclusion of DDGS was observed to increase ruminal concentrations of ammonia-N. Methane emissions were predicted to be greatest from LSH, but limit-feeding did not affect the C footprint in comparison to HAY.

In chapter 4, initial in situ DM disappearance was reduced for all forages tested in cows offered LSH or limit-fed DDGS (LDG) in comparison to HAY, but a positive associative effect was realized when SH and DDGS were limit-fed an isoenergetic mixture (MIX). The forage DM

disappearance depression observed in LSH and LDG was resolved within approximately 7 d. With the exception of bermudagrass, there was no difference between LSH and LDG in time to full recovery of ruminal forage DM digestibility. No statistical significance was observed for recovery time when forages were grouped by seasonality (cool-season or warm-season) or for perennial forages.

Overall, it may be inferred that limit-feeding co-product feedstuffs is a viable option for cows in yrs of adverse climatic conditions. This conclusion is drawn from the lack of adverse effects on cow or calf performance, digestive function, and the rapid recovery of ruminal forage digestive function when this system was implemented.