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USE OF DISTILLERS GRAINS IN PRECISION-FED DAIRY HEIFERS

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by

Francisco X. Suarez-Mena

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The thesis of Francisco X. Suarez-Mena was reviewed and approved* by the following:

Arlyn J. Heinrichs
Professor of Dairy and Animal Science
Thesis Advisor

Gabriela A. Varga
Distinguished Professor of Animal Science

Robert J. Van Saun
Professor of Veterinary Science

Daniel R. Hagen
Professor of Animal Science
Coordinator, Graduate Programs in Animal Science

*Signatures are on file in the Graduate School

ABSTRACT

Objectives of this study were to determine effects of feeding differing forage to concentrate ratios (**F:C**) and corn dry distillers grain with solubles (**DDGS**) inclusion rates on digestion, rumen fermentation and chewing activities, in precision–fed dairy heifer rations. A split plot design with F:C as whole plot and DDGS inclusion level as sub–plot was administered in a 4–period (19 d) 4 × 4 Latin square. Eight rumen cannulated Holstein heifers (12.5 ± 0.5 mo and 344 ± 15 kg, age and BW, respectively) housed in individual stalls were allocated to 2 F:C (50:50 **LF** or 75:25 **HF**; DM basis) and to a sequence of DDGS inclusion (0, 7, 14 and 21%; DM basis). Forage was a mix of 50% corn silage and 50% grass hay (DM basis). Diets were fed to allow for 800 g/d BW gain and fed 1X/d. Chewing behavior was visually monitored for 48 h at 5–min intervals. Rumen contents were sampled at –2, 0, 2, 4, 6, 8, 10, 12, and 20 h after feeding for pH determination. Total rumen evacuation was performed at –2 and 5 h after feeding. Statistical analyses were conducted using the MIXED procedure of SAS. DMI linearly decreased as DDGS increased (6.6 to 6.1 ± 0.1 kg/d; $P < 0.01$). LF rations had greater apparent digestibility (**AD**) of DM (66.7 vs. 63.2 ± 0.8%; $P = 0.02$) and OM (69.0 vs. 65.2 ± 0.6%; $P < 0.01$). AD responded quadratically for DM, OM, ADF and NDF with 14% DDGS inclusion level having the highest values. Rumen concentration of ammonia tended to be higher for HF (7.7 vs. 6.5 ± 0.4 mg/dL; $P = 0.07$) and tended to increase as DDGS increased (6.5 to 8.1 ± 0.6 mg/dL; $P = 0.08$). Nitrogen retention decreased with increasing levels of DDGS (40.9 vs. 27.9 ± 4.4 g/d; $P = 0.01$). Molar proportions (% of total VFA) of acetate tended to be greater for HF (65.8 vs. 64.0 ± 0.6%; $P = 0.07$) and

decreased as DDGS increased (65.4 to 63.9 ± 0.5%; $P < 0.01$); propionate increased as DDGS increased (18.8 to 20.6 ± 0.3 %; $P < 0.01$). Acetate to propionate ratio decreased as DDGS increased (3.5 to 3.1 ± 0.1; $P < 0.01$). Rumen protozoa count decreased as DDGS increased (24.4 to 11.9 ± 3.2 × 10^4/mL; $P < 0.01$). No differences were found for rumen pH, and bacterial CP flow estimated by purine derivatives. Time spent eating tended to be longer for HF (151 vs. 112 ± 14 min/d; $P = 0.09$) and was not different for DDGS inclusion. Ruminating time did not differ by forage leveland linearly increased as DDGS increased (421 to 450 ± 15 min/d; $P = 0.03$). Total chewing time tended to be longer for HF (593 vs. 516 ± 28 min/d; $P = 0.10$) and to increase linearly as DDGS increased (553 to 579 ± 23 min/d; $P = 0.09$). Wet rumen digesta weight (46.6 vs. 37.6 ± 2.2 kg; $P = 0.03$) and volume (51.5 vs. 41.5 ± 2.5 L; $P = 0.03$) were greater for HF. We found that nutrient AD responds quadratically to DDGS level. Ammonia concentration and molar proportion of propionate increased; while molar concentration of acetate, acetate to propionate ratio, and rumen protozoa number decreased with increasing levels of DDGS. LF rations had greater DM and OM AD. Total chewing time increased by the addition of DDGS and higher F:C. DDGS influenced ruminating time with no effect on eating time while F:C affected eating time. Higher F:C increased rumen digesta weight and volume. Forage and DDGS levels did not affect rumen pH. Moderate levels (14% of DM) of DDGS appear to be more suitable for use in dairy heifer rations yielding the highest digestibility, however with a decrease in nitrogen retention.

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Chapter 1

Introduction

The rearing of replacement dairy heifers represents a large expense to the overall farm operation. The objectives of heifer rearing programs are to provide the best quality heifer that would reach its maximum lactation productivity potential. At the lowest economical expense, without compromising the animal's welfare, and minimizing environmental impact.

Because feed represents the largest cost associated with raising heifer replacements (Gabler et al., 2000), control of total heifer rearing costs is essential to farm profitability. One of the strategies to reduce feed cost and control average daily gain is to limit the intake of nutrient dense rations; however, welfare of heifers under this management practice has been questioned (Kitts et al. 2011).

Pennsylvania dairy farmers use substantial amounts of corn for feeding, but the increasing use of corn for ethanol production has increased its price, and indirectly the price of other feeds, affecting farming economics. An option to reduce feeding costs is to include ethanol production byproducts such as dry distillers grains in the rations. Dry distillers grains have been successfully incorporated in large quantities for growing feedlot cattle rations (Klopfenstein et al., 2007), but little is known of its effects in rations limit fed to dairy heifers. Therefore, the objective of this research is to determine the effects of including corn distillers grains in rations for limit-fed dairy replacement heifers.

Chapter 2

Literature review

Precision-feeding the dairy heifer

Management of growing dairy heifers has changed over time. Traditionally, after heifers were weaned the time invested in their care was minimal, resulting in less than optimum management compared to today's standards. This, is because until the onset of lactation replacement heifers will not provide economic benefits to producers (Kitts et al., 2011; Zanton and Heinrichs, 2009). Nevertheless, objectives of rearing heifers have not changed over time. Swanson's definition (1967) "Develop in the heifer her full lactation potential at a desired age and at a minimum of expense" is similar to the definition of Hoffman et al. (2007) "To rear heifers at a low economic and environmental cost without compromising future lactation performance." The only difference is that Hoffman et al. (2007) stressed the importance of the environmental cost in their definition. In the future, the welfare of heifers likely will be included in definitions of heifer rearing objectives; therefore it is important to increase our knowledge in this area. Precision-feeding programs provide the heifer with the minimum amount of nutrients to reach the targeted average daily gain (**ADG**). Thus, controlling heifer growth and minimizing nutrient discharge to the environment.

Optimum growth rate for future productivity

The optimum growth rate for replacement heifers is directly related to the age at first calving (**AFC**); a high AFC increases the nonproductive life of the animal and rearing cost, while very low AFC reduces overall performance of the heifer (Roy, 1978). Body weight at calving (Hoffman and Funk, 1992), first lactation culling (Ettema and Santos, 2004), and prepubertal growth (Zanton and Heinrichs, 2005) also need to be taken into account. The suggested AFC that meets these parameters has not changed over the years, 23 to 24.5 months (Swanson, 1967; Heinrichs, 1993; Ettema and Santos, 2004). In order to achieve AFC before 20 mos, prepubertal growth needs to be accelerated so heifers can reach puberty at an earlier age (Swanson, 1967). However, this has been found to negatively affect first lactation milk production (Swanson, 1960; Radcliff et al., 2000), because it prevents normal development of the mammary gland (Swanson, 1960; Davis Rincker et al., 2008). In a meta-analysis by Zanton and Heinrichs (2005) the optimum average daily gain (**ADG**) for Holstein heifers between 150 and 320 kg of body weight (**BW**) that maximized first-lactation milk production was found to be 799 g/d. Postpubertal growth should also be controlled to avoid overconditioning at calving since it can be detrimental to lactation performance (Hoffman et al., 1996). Optimum growth can be achieved by restriction of good-quality feed (Swanson, 1967) or by feeding low-energy, high-fiber forages (Hoffman et al., 2007).

The economics of feeding dairy heifers

Costs of rearing replacement heifers account for 15 to 20% of total milk production cost and often represents the second greatest cost to the dairy farm, overcome only by the cost of lactating cows' feed (Heinrichs, 1993). Reducing expenditures on raising heifers may contribute to reducing whole-farm expenses (Zanton and Heinrichs, 2009). Feed costs represent over 60% of the total cost to rear a replacement heifer (Gabler et al., 2000). The high contribution of feed cost to total cost presents itself as an opportunity to search for alternative feed management practices and less expensive byproducts that reduce feed expenses.

Heifers traditionally have been fed high forage, low grain diets (Heinrichs, 1996) to avoid overfeeding or because of high grain cost. Concentrates have higher concentration of energy and protein so a reduced amount of dry matter (**DM**) in comparison with forages provides the animal with its nutrient requirements. Under this consideration, feeding concentrate could be more cost effective than feeding forage (Zanton and Heinrichs, 2009).

Feeding nutrient dense rations to dairy heifers

Restricting the intake of rations with higher proportion of concentrates allows for optimum growth of replacement heifers, with the added effects of improved feed efficiency and reduced manure excretion, without compromising future lactation performance (Zanton and Heinrichs, 2009).

In a study (Zanton and Heinrichs, 2007) to determine the effects of restricting a diet high in concentrates in prepubertal heifers, Holstein heifers starting at 125 kg of BW were fed either 75:25 or 25:75 forage to concentrate ratio (**F:C**) rations for the entire prepubertal period (245 d). Intake was controlled to allow similar ADG for both treatments and animals received one common postpubertal diet. Structural growth, ADG, and weight at puberty were not affected by treatment, and production was maintained. The animals from this study were followed though the second lactation and productive outcomes were not negatively affected by feeding the diet higher in concentrate during the prepubertal period (Zanton and Heinrichs, 2009). First lactation production outcome of heifers limit-fed high concentrate diets was confirmed by Lascano et al. (2009) in prepubertal heifers and by Hoffman (2007) in gravid heifers. Furthermore, a recent meta-analysis by Zanton and Heinrichs (2010) reached the same conclusions across a wide range of management types and daily gains.

Feed efficiency was increased by limit-feeding diets higher in concentrate to prepubertal heifers (Zanton and Heinrichs, 2007; Lascano et al., 2009), and gravid heifers (Hoffman et al., 2007). The efficiency advantage comes from the added effects of metabolic and digestive adaptations. The metabolic adaptations when animals are limit-fed a ration with a greater proportion of concentrates include a reduction in maintenance energy needs due to a reduction in the gastrointestinal tract mass (McLeod and Baldwin, 2000). This results in lower portal-drained viscera and liver heat production (Reynolds et al., 1991) and less heat production associated with eating and ruminating (Susenbeth et al., 1998). Digestive adaptations include greater DM digestibility (Reynolds et al., 1991;

Moody et al., 2007), reduced fecal, methane, and urinary energy loss (Reynolds et al., 1991), and increased retention time for limit-fed rations (Leaver et al., 1969).

Manure excretion is reduced in response to lower dry matter intake (**DMI**) when limit-feeding high concentrate rations to heifers (Moody et al., 2007; Hoffman et al., 2007; Lascano et al., 2009). A reduction in fecal output reduced the negative impact of the heifer on the environment (Moody et al., 2007). Careful considerations should be taken when assessing the extent to which manure reduction will lessen the impact of heifers on the environment when limit feeding because the amount of P and N excreted was not reduced (Hoffman et al., 2007), and neither were ammonia emissions (Lascano et al., 2008).

Chewing activities and welfare of precision-fed dairy heifers

The limit-feeding strategy has given rise to some welfare concerns for heifers reared under this management scheme. Redbo and Nordbland (1997) observed that limit feeding induces the development and increases the frequency of oral stereotypies in heifers. Stereotypies are movements regularly repeated in the exact same way that have no obvious function in the situation in which they are performed (Ödberg, 1978). In cattle these behaviors consist in repeated rolling of the tongue, bar-biting and biting/licking of the stall (Redbo, 1990). In cattle stereotypies may be triggered by frustrated feed manipulation (Redbo, 1992), heifers spent less time eating and ruminating when limit-fed (Redbo and Nordblad, 1997). Also, stereotypic behavior increased in heifers housed in tie stalls (Redbo, 1992; Krohn, 1994). Broom (1983) considered the occurrence of prolonged

stereotypies to be indicators of poor animal welfare. Redbo (1990) observed that in heifers stereotypies can be trigged by frustrated feeding and hunger; frustration accompanied by anxiety is what causes stereotypies (Ödberg, 1978).

Less time spent eating and ruminating may reduce saliva secretion, because rate of secretion is greater while chewing than resting; Bailey and Balch (1961) observed that saliva secretions from one parotid gland of a small steer for eating, ruminating and resting were about 20, 25 and 10 mL/min respectively. Less saliva secretion would increase the risk of acidosis, since the buffering capacity of saliva is one of the factors that determines rumen pH (Krause et al., 2002).

Dairy cattle spend 4 to 7 and 5 to 9 h/d eating and ruminating respectively (Beauchemin, 1991), but the time heifers spend eating and ruminating in confined environments has a greater range. Many factors affect these behaviors, among them: level of feed intake, ration composition, forage quality and length, and feeding method (Beauchemin, 1991). Prepubescent heifers limit-fed to 2.02% of BW spent 1.2 h/d eating when offered a 40:60 F:C total mixed ration (**TMR**); when straw was added (to constitute about 30% of feed) either on the side or in the TMR, time spent eating increased to 3.4 and 4.8 h/d respectively (Kitts et al., 2011). Feeding method also affected time spent eating of prepubescent heifers fed ad libitum grass hay with a fixed amount of concentrate at about 62:38 F:C, either separated, with grain top dressed, or as a TMR. Heifers spent 2.3, 2.8 and 3.1 h/d eating respectively (DeVries and von Keyserlingk, 2009). As in the trial with limit-fed heifers (Kitts et al., 2011), the addition of straw increased eating time of ad libitum-fed prepubescent heifers, from 3 h/d when fed a 69:31 F:C TMR, to 3.3 h/d when straw (20% of DM) was added to the TMR (Greter et al.,

2008). Lower eating time may be compensated by an increase in ruminating time, which may add to similar total chewing time, unfortunately time spent ruminating was not determined in the aforementioned trials.

Jaster and Murphy (1983) fed heifers (340 kg BW) 3 lengths of hay ad libitum; as particle size decreased, time spent eating decreased, and fewer boluses were processed per minute of ruminating, while total chewing time was similar. Thus, heifers eating the finer diets may have compensated for less time spent eating by increasing the time spent ruminating each bolus. On average, heifers spent 8.5 and 8.2 h/d eating and ruminating, respectively. Eating rate increases when animals are limit-fed, lowering eating time; this may be compensated for by a decrease in chewing rate while ruminating. Metz (1975) observed greater rumination time per kg of intake as intake decreased in limit-fed, non-pregnant, non-lactating cows fed long hay; however, when cows were fed hay wafers rumination time increased linearly as DMI increased. Conflicting results may be related to the effect of forage particle length on rumination; also, mature steers fed corn silage 90:10 F:C did not reduce rumination time per kg of intake when DMI was 65% higher (Sudweeks et al., 1980). These findings suggest that there might be an interaction between intake level and particle length and quality of forage on rumination rate.

Increasing feeding frequency was proposed as a strategy to reduce the risk of ruminal acidosis by means of stabilizing ruminal conditions when feeding high concentrate diets (Soto-Navarro et al., 2000); however, when heifers (385 ± 6.2 kg BW) had ad libitum access to straw and concentrate fed 1 to 4 times a day, average and lowest pH, and volatile fatty acids (**VFA**) concentration and proportions were not affected (Robles et al., 2007). Steers (344 ± 26 kg BW) in Soto-Navarro et al. (2000) were limit-

fed to 90% of ad libitum consumption of a 90% concentrate ration; the researchers observed a tendency for daily mean pH to be lower when feeding once vs. twice daily. This is in contrast with Yang and Varga (1989) who observed minimum pH to be lower when feeding frequency was increased in lactating dairy cows. Robles et al. (2007) offered grain and forage free choice to heifers and intake consisted of 90:10 F:C, the same ratio as in Soto-Navarro et al. (2000). Feeding frequency did not affect daily percentages of time spent eating, ruminating, resting, or licking (Robles et al., 2007). These results contrast with Welch and Smith (1969) who observed rumination rate decreased when hay was fed as a single meal vs. continuously to fasted sheep. Slower rumination rate would increase total rumination time, increasing the saliva output and buffering the rumen.

Kitts et al. (2011) suggested that limit-fed heifers are at greater risk of lameness because of long inactive standing time and risk of subacute ruminal acidosis. In their study heifers fed the ration with lower F:C (more restricted) spent more time standing inactively, this observation is in agreement with the report of Hoffman et al. (Hoffman et al., 2007). Greater time standing increases the risk of laminitis in lactating cows, (Nocek, 1997; Cook et al., 2004). Nocek (1997) associated the risk of subacute ruminal acidosis caused by increased consumption of highly ruminally available carbohydrates as a risk factor for lameness. However, heifers in the Kitts et al. (2011) study spent the same total time standing (inactive standing plus standing while eating). It can be speculated that heifers in the lower F:C were inactively standing due to the presence of feed in adjacent pens. Intensified stereotypic behavior was observed in pigs that could see feed but could not reach it (Rushen, 1985). The relationship between high concentrate diets and ruminal

acidosis is well established in lactating dairy cows (Nocek, 1997), but results in growing heifers fed such diets where average and lowest pH did not reach critical levels (Moody et al., 2007; Robles et al., 2007) suggest that growing heifers can better tolerate high concentrate diets.

An increase in vocalization time was observed in heifers limit-fed to 80% of ad libitum DMI, but only during the first 5 wk after treatment allocation (Hoffman et al., 2007), which suggests that this behavior lasts only while heifers adapt to the new dietary regimen. Vocalization scoring was suggested as an indicator of welfare during cattle slaughter (Grandin, 1998), but this does not specify vocalization type so the quality and degree of impairment remain unknown (Manteuffel et al., 2004). Vocal behavior in cattle is poorly understood (Watts and Stookey, 2000).

Conclusions about the welfare of heifers reared under this management cannot be reached because of limited literature and many factors interacting with behavior in limit-fed heifers. It is important to increase our knowledge of animal behavior as media attention continues to look at animal systems and their effects on animal welfare. Further research on the welfare implications of limit feeding heifers will help address and understand any possible concerns of these systems.

Corn dry distillers grains

The rising demand of grains for the production of biofuels has dramatically increased commodity prices, corn in particular (Schmit et al., 2009). The inclusion of low cost by-products from the production of biofuels may help to reduce the impact of high

priced corn on feeding cattle. As ethanol production increases and thus the availability of distillers grains (**DG**), it may be more economically feasible to include DG as the primary source of supplemental nutrients in forage-based heifer diets (Martin et al., 2007). Corn fermentation residue in the distilling industry can have the coarse grains separated and dried to produce distillers dried grains (**DDG**) or have only water removed to produce distillers dried grains with solubles (**DDGS**) (Waller et al., 1980). Wet distillers grains with solubles (**WDGS**) or without solubles (**WDG**) are fed in facilities located in close proximity to the milling plants, including feedlots. Even though it may seem counterproductive to use energy for drying DG, it proves necessary to facilitate transportation to destinations far from milling plants (Klopfenstein et al., 2008). The focus of this review will be on the inclusion of DDGS in replacement heifer diets; however, relevant research on other DG products and animals fed to meet other purposes than replacing the herd will be included.

Nutrient composition

Nutrient composition of DG has changed over time due to the increased fermentation efficiency of ethanol plants, and therefore protein and energy levels may be higher than the NRC (2001)values (Schingoethe et al., 2009). Although most distillers grains available today is as DDGS (Schingoethe et al., 2009) continuing changes in ethanol plants result in new DG products with different nutrient composition; some processes extract part of the fat of DG, and others remove the germ and/or bran of corn before fermentation (Mjoun et al., 2010). Starch comprises about two-thirds of corn, so

after starch is fermented to ethanol, the one-third of nutrients left will have a threefold concentration of the initial values. This represents about 30% crude protein (**CP**), 12% fat, 36% neutral detergent fiber (**NDF**), and 0.9% P of DDGS DM (Klopfenstein et al., 2008). Zein, the primary protein of corn DG, is only partially degraded in the rumen (Klopfenstein et al., 2008) thus DG are a good source of rumen undegradable protein (**RUP**), with values ranging between 47 and 64% of CP concentration (Schingoethe et al., 2009). When compared to soybean meal (**SBM**), the typical feed against which other protein sources are evaluated, DGGS amino acid (**AA**) flow to the duodenum of lactating cows was higher (Santos et al., 1984). Digestibility of RUP in DDGS is assumed by NRC to be 80% (NRC, 2001), but now known to range from 84 to 91% (Mjoun et al., 2010). The increase in RUP digestibility of DDGS may be due to improvements in the ethanol industry processing that minimizes heat damage; this improvement also could be the reason for an increase in lysine concentration from 2.24% of CP in the latest NRC (2001) to 3.15 % of CP (Schingoethe et al., 2009). Lysine is susceptible to heat damage via the Maillard reaction (Choi et al., 1949). An increase in lysine concentration would be beneficial because of the low levels found in corn-based CP (Hollmann et al., 2011). Lysine has been shown to be the first-limiting AA for milk yield and milk protein yield in dairy cows (Schwab et al., 1992).

Energy of DG may be underestimated in the NRC (2001). Birkelo et al. (2004) estimated 3.36 Mcal/kg of metabolizable energy (**ME**) in WDGS, an 11% increment of the 3.03 Mcal/kg of ME in DDGS published in the NRC (2001). This difference may be due to improvements in the distilling process rather than energy losses in the drying process (Birkelo et al., 2004; Schingoethe et al., 2009). Energy in DG is largely found in

the fat component and highly digestible fiber of this by-product (Schingoethe et al., 2009; Gehman and Kononoff, 2010). High digestibility of NDF was reported by Birkelo et al. (2004), who observed 61% WDGS NDF digestibility in lactating cows, and Vander Pol et al. (2009), who observed 71% and 79% NDF ruminal and total-tract digestibility in Holstein steers. Fiber digestibility of DG is high because of their low lignin content (NRC, 2001). The small particle size of DG may provide a greater surface area for microbial attachment, which may make NDF readily fermentable energy for rumen microbes. In contrast Ham et al. (1994) suggested high digestibility of DG fiber due to post-ruminal digestion. Similarly it was suggested that some of the oil content of DG is resistant to rumen hydrogenation, thus allowing it to be directly utilized in the small intestine and increasing its digestibility (Klopfenstein et al., 2008). This is supported by the work of Vander Pol et al. (2009), who observed greater digestibility of fat in WDGS when compared with corn oil. However, high fat content in some DG may restrict its inclusion level because high proportions of fat in the ration can reduce DMI and ruminal fermentation (NRC, 2001)

Adding solubles to DG slightly decreases CP content and increases fat, P and S contents, while drying increases RUP supply (Cao et al., 2009). The amount of solubles blended with the DG and the fermentation efficiency of the processing plant affect the nutrient composition of the DG; for this reason it is important to obtain analytical data of the specific product received and for suppliers to provide uniform and standardized DG (Schingoethe et al., 2009).

Distillers grains in heifer rations

Distillers grains have been traditionally fed as a protein source for cattle (Ham et al., 1994) and in combination with urea can effectively replace SBM in growing cattle without reducing performance (Waller et al., 1980). Today, DG may be included not only as a protein source, but to provide most of the supplemental nutrients in forage-based heifer replacement rations (Martin et al., 2007) or as the primary energy source by the feed-lot industry (Depenbusch et al., 2009a).

Utilization of DG by the feedlot industry is vast, thus research of the inclusion of DG in feed-lot cattle is abundant. The DG level of inclusion that maximized DMI, ADG and final BW in feed-lot finishing-diets was 15% in work by Depenbusch et al. (2009a). Research from the same lab compared 13 and 15% DDGS levels with several diets at 0% DDGS inclusion, and observed no differences in feed efficiency (Depenbusch et al., 2008; Depenbusch et al., 2009b). Other researchers observed ADG and feed efficiency to improve at greater inclusion levels of DG in finishing diets; 40% in Ham et al. (1994) and up to 50% in Firkins et al. (1985). The increased feed efficiency when feeding DG may be due to a reduction in subacute ruminal acidosis because of lower starch content when compared with corn-based diets Firkins et al. (1985).

Although research coming from the feedlot industry is valuable, the objectives are very different when compared to raising replacement heifers. Replacement heifer growth has to be controlled (NRC, 2001; Zanton and Heinrichs, 2005), while feedlots target maximum growth in minimum time. To date little has been published on the inclusion of

DG in replacement heifer diets, and at the time of this review none were found in heifers fed to a restricted DMI.

Martin et al. (2007) investigated effects of DDG as an energy source in growth and reproduction of beef heifers with ad-libitum access to hay. Supplementing DDG vs. an isocaloric diet differing in protein digestibility did not affect BW, ADG or body condition scoring (**BCS**); artificial insemination (**AI**) conception and pregnancy rates improved in heifers fed DDG. In contrast Harris et al. (2008) observed that ADG improved and found no differences in reproduction parameters when comparing supplementation of DDG vs. whole raw soybeans in diets similar in energy and CP fed to beef heifers. The effect of DDG on ADG fed to grazing beef heifers was studied by MacDonald et al. (2007). In this study, supplements with relative contributions of RUP and fat to DDG, were compared with DDG and corn bran. Heifer ADG was higher for the DDG and the supplement with relative RUP treatments. The ADG increase of the heifers fed the supplement with relative RUP was 39% of that for DDG, this increase was interpreted as the gain related to the RUP of DDG. Although the supplement with fat content relative to DDGS did not provide additional gain, fat in combination with the NDF content should be responsible for the additional gain in the DDG treatment.

In situ fiber digestibility was compared in heifers with ad libitum access to grass hay and supplemented with either dry rolled corn (**DRC**) or DDGS concentrates to about 18% of total DMI (Loy et al., 2007). Disappearance rate of hay NDF was faster for the DDGS treatment (4.09 vs. 3.43 %/h), while pH was not affected. Also in Loy et al. (2007), the ratio of purine derivatives to creatinine in urine (**PD:C**), an indicator of

bacterial CP flow, was higher for the DDGS treatment so DDGS favored rumen microbial growth.

One concern with feeding high levels of DDGS is the risk of approaching the maximum recommended level of sulfur intake, even more so if water is high in sulfur. High levels of sulfur in DG, as much as 1% in some cases, come from the sulfur-containing compounds used to control pH and clean equipment in the ethanol plants (Klopfenstein et al., 2008; Schingoethe et al., 2009). Excess sulfur could cause a polioencephalomalacia syndrome, and may reduce feed intake and performance (NRC, 2001). Phosphorus and nitrogen may be overfed at high intakes of DG, mostly when fed as an energy rather than protein source (Klopfenstein et al., 2008). Overfeeding protein will result in an increased amount of nitrogen in manure, this would increase ammonia emissions and groundwater contamination, both detrimental to the environment (VandeHaar and St-Pierre, 2006). Increased phosphorus concentration in manure as result of high DG intake may also represent a liability for the waste nutrient management of some farms (Schmit et al., 2009). Phosphorus wastes from agriculture accelerate the eutrophication of lakes and streams and are major environmental concerns (VandeHaar and St-Pierre, 2006).

The inclusion of DDGS from the production of biofuels may help to reduce the impact of high priced feed on feeding cattle; however DDGS have not been evaluated in limit-fed heifer rations. Therefore, there is a need for research that determines the effects of including DDGS in limit-fed replacement heifer rations, on DM digestibility, rumen fermentation, and nutrient output.

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Chapter 3

Evaluation of corn dry distillers grains in precision-fed heifers

Introduction

The rising demand of corn for the production of ethanol has been a contributing factor to the dramatic increase in feed prices (Schmit et al., 2009). An alternative to reduce feeds costs is to include by-products of ethanol production in rations. Distillers grains with solubles, the major byproduct of ethanol production, have been successfully fed as a major feed ingredient for feedlot cattle (Klopfenstein et al., 2008), and as a supplement for beef replacement heifers (Martin et al., 2007).

Restricting intake of nutrient dense rations to dairy replacement heifers permits control of their growth, and can reduce feed costs and nutrient waste (Zanton and Heinrichs, 2009b). However, welfare of heifers reared under this management has been questioned because of behavioral changes (Kitts et al. 2011).

Reducing feed cost would reduce the total cost of raising replacement dairy heifers because feed is the largest expense associated with it (Gabler et al., 2000). The added effects of precision-feeding and inclusion of distillers grains in heifer diets could further reduce feed costs.

Objectives of this experiment were to evaluate effects of including corn dry distillers grains with solubles in diets fed at two levels of F:C, for heifers reared under precision-feeding management.

Materials and methods

Animals and feeding

All procedures involving the use of animals were approved by the Pennsylvania State University Institutional Animal Care and Use Committee. Eight Holstein heifers were surgically prepared with a rumen cannula (7.62 cm i.d.; Barr Diamond, Parma, ID) under local anesthesia, 2 mo prior to the beginning of the experiment and later refitted with larger cannulae (10.16 cm i.d.; Barr Diamond, Parma, ID). Heifers (12.5 ± 0.5 mo and 344 ± 15 kg, age and body weight (**BW**) respectively, at the beginning of the experiment) were randomly assigned to a split-plot 4 × 4 Latin square experimental design. Whole plot was forage to concentrate ratio (**F:C**) either 50:50 (**LF**) or 75:25 (**HF**) on a dry matter (**DM**) basis, and subplot was level of inclusion of corn dry distillers grains with solubles (**DDGS**), either 0, 7, 14 or 21% DM basis. Forage was a mix of 50% corn silage (**CS**) and 50% grass hay on a DM basis. Experimental periods were 19 d in length with 14 d for adaptation and 5 d for sampling. Heifers were housed in individual tie-stalls (117 × 302 cm) with rubber mat flooring, in a mechanically ventilated barn with continuous access to fresh water. Lighting was controlled by an automated system to allow for 13.5 h/d, except on intensive sampling days when light was provided for 24 h. During non sampling days heifers were let out in the outdoor exercise lot for 3 to 4 h/d prior to feeding, BW was recorded on their way in and out of the exercise lot. Rations were balanced to provide equal amounts of nutrients and targeted to allow for 0.8 kg of average daily gain (**ADG**). The amount of feed offered was adjusted weekly based on BW; except the week before and during sampling. Single batches of grain ingredients were bought and stored to provide for the length of the experiment.

Grain ingredients and a mineral-vitamin premix were mixed for each treatment at the beginning of each experimental period, in a drum mixer (Calan Super Data Ranger, American Calan, Northwood, NH); forages were mixed daily using the same equipment. The forage mix, grain mix, and NPN source of each ration were hand mixed (because amounts were too low for mixer) and delivered once daily at 1200 h.

Samples and analyses

Grain mix for each diet was sampled at the beginning and end of each experimental period, and composited by diet at the end of the experiment. Forage was sampled daily during collection days; a subsample was frozen at -20 °C for later analysis, and 2 other subsamples were immediately sieved for particle size determination in the Penn State Particle Separator (PSPS) and the ASABE particle separator (ASABE, 2007).

Feces and urine were completely collected from d 14 immediately after feeding to d 18 immediately before feeding for 4 d of total collection. Urine was collected using a noninvasive urinary device (Lascano et al., 2010), connected to a container with distilled water and 12 N HCl to maintain pH below 3 and minimize ammonia ($\mathbf{NH_3}$) volatilization. The distilled water was added to avoid the formation of precipitates. Feces were collected hourly from vinyl covered boards on the floor and stored in airtight containers. Every 24 h, urine and feces were weighed, mixed, and sampled. Urine samples were immediately frozen at -20 °C. Feces were stored at 4°C until the last day of that collection period, then composited by period proportionally to daily output; one sub-sample was dried and a fresh sub-sample was frozen at -20 °C.

Forage mix and feces were dried in a forced air oven at 65 °C for 48 h and ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). After drying and grinding forage was composited by period. Fecal, grain mix, and forage mix composited samples were analyzed for DM, ash, and crude protein (**CP**) (AOAC, 1990), neutral detergent fiber (**NDF**), and acid detergent fiber (**ADF**) (Van Soest et al., 1991). Frozen fecal samples were thawed and analyzed for CP according to the procedure detailed above. Urine samples were analyzed for N (AOAC 1990) and purine derivatives (Chen and Gomes, 1992). Feed was analyzed for starch content by the procedure of Bach Knudsen (1997), with the modifications discribed by Zanton and Heinrichs (2009b). Degradable protein was analized as discribed by Krishnamoorthy et al. (1983).

Rumen contents were collected from dorsal, ventral, cranial, caudal, and medial areas of the rumen at -2, -1, 0, 1, 2, 4, 6, 8, 10, 12, 16, 20 and 24 h after feeding on d 17. Rumen contents were mixed thoroughly, sampled, and strained through 2 layers of cheesecloth; fluid was immediately analyzed for pH using a handheld pH meter (HI 98121, HANNA instruments, Woonsocket, RI). For rumen NH_3 and volatile fatty acids (**VFA**) analysis, 15 mL of filtered rumen fluid were placed into bottles containing 3 mL of 35% metaphosphoric acid and 3 mL of 0.6% 2-ethyl butyric acid (internal standard) and frozen at -20 °C until analyzed. After thawing, rumen fluid was centrifuged 3 times at 4,000 × g for 30 min at 4°C to obtain a clear supernatant, which was analyzed for NH_3 according to Chaney and Marbach (1962) and VFA molecular concentration by gas chromatography (Yang and Varga, 1989). For rumen protozoa number estimation, 5 mL of rumen fluid was combined with 5 mL of methylgreen-formalin-saline (MFS) solution (Ogimoto and Imai, 1981) and stored in darkness at 4°C. Counting was done using a Fuchs Rosenthal counting chamber (#3720, Hausser Scientific, Horsham, PA) as in Hristov et al.

(1999). Each sample was counted twice and if either value differed by more than 10% from the average, an additional count was done. Whole rumen evacuations were done at -2 and 4 h after feeding at the end of each experimental period, to determine the rumen digesta weight and volume.

Physical effective NDF (**peNDF**) of diets, was determined by multiplying its NDF concentration and the proportion of particles retained on the 1.18 mm sieve (Mertens, 1997) of the ASABE particle separator (ASABE, 2007). The proportion of particles retained on the 1.18 mm for each ration was obtained by the addition of the forage and grain fractions which were sieved separately.

Chewing behavior was visually monitored for 48 h at 5-min intervals by a team of trained individuals. Five categories were recorded: eating, ruminating, idle, licking, and other. Each activity was assumed to persist for the entire 5-min interval. Oral stereotypies (tongue-rolling, biting, chain-chewing, and licking) and self grooming were recorded as licking. Total chewing was calculated by adding eating and ruminating behaviors.

Statistical analysis

All statistical analysis were conducted in SAS (Version 9.2, SAS Institute, Cary, NC) using the MIXED procedure. Data were analyzed as a split-plot, Latin square design with fixed effects of period, forage, DDGS level, and forage × DDGS level interaction, and a random effect of heifer (forage). Because of unequally spaced rumen sampling, mean pH, VFA, and NH_3 concentrations were determined by calculating the area under the response curve according to the trapezoidal rule (Shipley and Clark, 1972). The sequences of forage and DDGS level were

balanced for carryover with respect to previous forage and DDGS level such that all treatments followed every other treatment once; therefore, fixed effect of previous treatment was included in the analysis. Fixed effect of time and its interaction with other fixed effects was included in the model when multiple observations occurred in a day, except for chewing activities where only daily averages were analyzed. Variance homogeneity was evaluated for the main effects of forage and DDGS level and time relative to feeding when repeated measures were analyzed; evidence of significant heterogeneity was determined using the Levene test for equality of variance. Least square means are presented in tables and evidence for statistical significance was declared at $P < 0.05$.

Results and discussion

Dietary nutrient composition and intake

Nutrient composition of diets is presented in Table 1 and intake in Table 2. Rations were formulated and fed with the objective of providing similar amounts of energy and N. Dietary NDF and ADF were higher for HF, as was their intake due to the higher level of forage in these rations. Fat concentration in the diet increased with the addition of DDGS, and as a consequence so did metabolizable energy (**ME**) concentration; therefore, diet DMI decreased as more DDGS was added. When ME intake was calculated with the actual digestibility of diets, its intake decreased as more DDGS were added to the rations, although differences were minor. Similarly N intake (**NI**) slightly decreased as DDGS concentration increased. Neither ME nor N daily intakes differed by F:C when analyzed as g/d or Mcal/d, but both where greater for LF when analyzed as intake per kg of $BW^{0.75}$; although differences were marginal. NI approached the

level recommended by Zanton and Heinrichs (2009a) for optimum N utilization in precision-fed dairy heifer rations (1.67 g/kg of $BW^{0.75}$). Daily DMI was not different for F:C when analyzed as kg/d, as intake was adjusted to BW which added variability, therefore when analyzed as g/kg $BW^{0.75}$, DMI was higher for HF. Higher DMI for HF were also reported in experiments that controlled nutrient intake at different F:C (Hoffman et al., 2007; Lascano and Heinrichs, 2009; Lascano et al., 2009; Zanton and Heinrichs, 2009a); because the lower nutrient density in HF rations is compensated for by increasing DMI (Hoffman et al., 2007).

Digestibility

Apparent digestibility (**AD**) of nutrients is presented in Table 3. The AD of DM (Figure 1) and organic matter (**OM**; Figure 2) was greater for LF rations. Similar observations have been reported for limit-fed dairy (Hill et al., 2007; Lascano et al., 2009; Zanton and Heinrichs, 2009a; Lascano and Heinrichs, 2011) and beef heifers (Reynolds et al., 1991). Greater digestibility of LF rations can be attributed to an increased retention time in the rumen of rations with lower F:C (Leaver et al., 1969; Colucci et al., 1990). Digestibility of ADF (Figure 3) and NDF (Figure 4) was not different for F:C, this conflicts with other studies where fiber digestibility was greater for lower F:C. Zanton and Heinrichs (2009a) observed greater NDF, but similar ADF, digestibility for lower F:C, and concluded that hemicellulose was better digested in rations with lower F:C. Lascano and Heinrichs (2011) observed similar hemicellulose digestibility but greater cellulose digestibility at lower F:C; NDF and ADF digestibility decreased for higher F:C. Forage sources were different between the two studies, which may account for differences in fiber fraction digestibility. In the present study F:C difference between treatments was 25 percentage

units, less than in Lascano and Heinrichs (2011) and Zanton and Heinrichs (2009a) where F:C difference was 60 and 50 percentage units respectively. Narrower treatment differences in the present study may be the reason for no statistical differences in fiber digestibility. Nitrogen apparent digestibility (**NAD**; Table 4; Figure 5) was not different for F:C, similar to previous studies (Moody et al., 2007; Lascano et al., 2009; Lascano and Heinrichs, 2011). In contrast, Hill et al. (2007) and Zanton and Heinrichs (2009a) observed higher N digestibility at lower F:C. Zanton and Heinrichs (2009a) attributed the improved N digestibility of LF diets to greater levels of N excreted in the feces of HF, which agrees with Hill et al. (2007). In the studies where NAD was similar, N excreted in feces and urine was also similar (Moody et al., 2007; Lascano et al., 2009; Lascano and Heinrichs, 2011); N retention was greater only in the study of Moody et al. (2007) because of greater N intake (NI). In the present study urine N excretion tended ($P = 0.08$) to be higher (6 g/d) for LF, equivalent to the numerical ($P = 0.13$) 7 g/d of higher NI for LF, therefore retained N was similar. Conflicting results in NAD could be due to NI level; Zanton and Heinrichs (2009a) observed a tendency ($P = 0.10$) for NI to interact with F:C in CP AD.

There was a quadratic AD response to increasing levels of DDGS for DM, OM, ADF, and NDF, with 14% DDGS the level at which AD was greatest. Depenbusch et al. (2009) fed increasing levels of DDGS (0, 15, 30, 45, 60, and 75%, DM basis) in the diets to beef heifers and observed a quadratic effect for DMI, ADG, and final BW, maximized at 15% DDGS. The quadratic effect in the present study and Depenbusch et al. (2009) could be in response to ether extract (**EE**) increasing with DDGS. When fat levels in the diet are low, rumen fermentation is not affected because rumen microbes are able to saturate fatty acids (**FA**), but at higher levels this capacity can be exceeded and FA can accumulate in the rumen and interfere with fermentation (NRC, 2001). When high levels of fat interfere with rumen fermentation,

digestibility of nonlipid energy sources is reduced (Jenkins, 1993). Fat digestibility was observed to respond quadratically to fat % in the ration by Wu et al. (1991), who observed higher FA digestibility when fat was added from 0 to 3% DM but lower digestibility when added to 6% of DM in mid-lactation dairy cow diets. This phenomenon may have in part caused DM and OM digestibility to respond quadratically to the addition of DDGS, increasing along with the addition of DDGS up to 14 % inclusion, but decreasing at 21 % DDGS because of the decrease in digestibility.

In the present study a quadratic interaction was observed for ADF and NDF AD; DDGS effects on AD were greater at lower F:C. Also, OM AD was lower at higher F:C but this difference diminished as DDGS level linearly increased. These interactions could be because the adverse effects of fat are greater in LF rations (Grant and Weidner, 1992). Higher digestibility at lower levels of DDGS (before EE concentration affects fermentation) could be due to the high NDF digestibility of DG. Fiber digestibility of DG is high because of their low lignin content (NRC, 2001).

Rumen fermentation and microbial population

Rumen fermentation parameters are presented in Table 5, and estimated bacterial CP flow and protozoa population in Table 6. Rumen pH was similar for F:C and DDGS. Rumen NH_3 concentration tended (P = 0.07) to be higher for HF, likely because of lower microbial populations. Estimated bacterial CP flow was numerically greater for LF (339 vs. 256 g/d; P = 0.39). Ammonia is used by bacteria for protein synthesis (NRC, 2001), and a decrease in their population would reduce NH_3 uptake and increase its concentration in the rumen. When protein

degradation exceeds microbial capacity to assimilate amino acids and NH_3, NH_3 accumulates in the rumen (NRC, 2001). The assimilation of N by bacteria depends on the supply of fermentable carbohydrates (Nocek and Russell, 1988; Bach et al., 2005), and the synchrony at which N and carbohydrates become available determines microbial growth (Bach et al., 2005). In the present study HF diets had more rumen degradable protein (**RDP**) and soluble CP and less non fiber carbohydrates (**NFC**) than LF diets (Table 1), so synchrony of nutrient availability of HF may have negatively affected microbial synthesis. The differences in VFA molar proportions in the present study although marginal, suggest a shift in bacterial species population. The greater proportion of acetate for HF is congruent to previous reports in limit-fed heifers (Lascano and Heinrichs, 2009) and steers (Sudweeks, 1977) and corresponds to a greater population of cellulolytic bacteria. Acetate results from the fermentation of structural carbohydrates by cellulolytic bacteria, while propionate results from the fermentation of non-structural carbohydrates by amylolytic bacteria (Enjalbert et al., 1999). The decrease in acetate proportion in LF diets is compensated for by an increase in propionate when the total concentration of VFA remain similar; so acetate to propionate ratio (**A:P**) decreases at lower F:C (Lascano and Heinrichs, 2009). In the present study propionate proportion and A:P were similar for F:C, so the increase in acetate proportion in HF was compensated for by a decrease of the isoacids. Higher isoacids in LF suggest a greater population of amylolytic bacteria. Isoacids are a product of branch-chained amino acid (**AA**) deamination (Lascano and Heinrichs, 2009). Protein deamination in the rumen and the consequent release of isoacids decreases when rumen degradable protein availability is low (Mansfield et al.,1994;Yang et al., 2004).

Rumen NH_3 concentration had a linear tendency (P = 0.08) to increase as DDGS levels increased; in spite of the marginal, but statistically significant linear NI g/kg $BW^{0.75}$ decrease

with increasing levels of DDGS. This could be a response of dietary non protein nitrogen (**NPN**) supply added along with DDGS levels to balance NI. The NPN fed was a controlled-release urea product (Optigen; Alltech Inc., Lexington, KY). Urea rapidly hydrolyzes to NH_3 in the rumen resulting in a peak concentration of ruminal NH_3 within 1 h after feeding (Taylor-Edwards et al., 2009). Optigen has a vegetable oil coating (Inostroza et al., 2010) that protects it from ruminal degradation and controls N release (Garcia-Gonzales et al., 2007). However, the only time point at which NH_3 concentration was different for DDGS level in this study was 2 h after feeding (Figure 1). Canola meal, the ingredient that was replaced by DDGS, has more rumen degradable protein than DDGS (NRC, 2001). There is also the possibility that NH_3 release was similar across DDGS levels but because ME intake linearly decreased with the addition of DDGS, microbial use of NH_3 was negatively affected and it accumulated in the rumen. The effect of lower ME intake as DDGS increased could have been exacerbated by a greater proportion of energy coming from EE as DDGS increased, providing less carbohydrates for bacteria. Also, the effect of lipids on bacterial growth is generally negative (Doreau and Ferlay, 1995). Total VFA concentration decreased linearly as DDGS level rose, which suggests that bacteria population declined by the addition of DDGS. However, this is not supported by the estimated bacterial CP flow (Table 6), which remained similar for DDGS levels. Molar proportions of acetate decreased and propionate increased linearly as DDGS levels increased; consequently A:P decreased with increasing levels of DDGS. Butyrate, valerate, and isobutyrate were unaffected by DDGS level, while isovalerate had a quadratic trend ($P = 0.08$) and a quadratic interaction with forage level. The observations for total VFA, proportions of acetic, propionic and butyric, and A:P are comparable to those reported by Leupp et al. (2009), who fed steers ad libitum diets containing 30:70 F:C and increasing levels of DDGS (0, 15, 30, 45, and 60% of DM). Lower A:P ratio can

be in response to a reduction in digestibility of nonlipid energy sources at greater EE intakes (Jenkins, 1993), possibly related to lower protozoa population as fiber digestion is greater in their presence. Mendoza et al. (1993) reported a decreased A:P ratio as response to defaunation in sheep. Linear interactions of propionate and A:P that were affected to a greater degree in LF could be due to the numerically greater bacteria population in LF and different species in HF vs. LF. These fermentation changes suggest that bacteria species populations changed with levels of DDGS. Previous studies have reported changes in bacterial population in response to DDGS. Callaway et al. (2010) utilized a quantitative molecular method (bacterial tag-encoded FLX amplicon pyrosequencing) and observed that ruminal *Succinivibrio* genera proportions decreased with the addition of DDGS. Fron et al. (1996), utilizing in vitro and in vivo techniques, suggested that distillers byproducts increase the relative numbers of lactic acid utilizing bacteria. Because of the confounding effects of less ME and more EE with increasing levels of DDGS, it remains unclear if one of them, the added effect of both, or another nutrient coming from DDGS is responsible for the changes in ruminal fermentation.

Rumen protozoa population responded quadratically to DDGS level; maximal population was at the 7% DDGS level. A large difference in protozoa occurred between the 14 and 21% DDGS level, with the later having about half the protozoa population of the first. The increase of EE along with DDGS is likely the reason for protozoa population decline, since fat negatively affects rumen protozoa concentration (Jenkins, 1993; Doreau and Ferlay, 1995). The large drop in protozoa numbers from the 14 to 21% DDGS levels may in part be responsible for the lower fiber digestibility at the 21% level; the digestion of fiber is greater in the presence of protozoa (Demeyer, 1981; Orpin, 1984).

Nitrogen dynamics

Nitrogen intake, AD, and dynamics are presented in Table 4. As discussed earlier, F:C had no effect on NAD or retention, and the increased N excreted in urine for LF seems to be responsive to higher NI. Level of DDGS did not affect NAD, but N retention decreased as DDGS level raised. Lower N retention was due to the linear increase of N excreted in urine, because N excreted in feces linearly decreased as DDGS levels increased. As mentioned earlier, this is likely a result of the NPN added to rations along with DDGS and a lack of synchrony of N and energy available for microorganisms in the rumen. It appears that substantial proportions of the NPN rapidly hydrolyzed to NH_3 in the rumen; where it was absorbed, metabolized to urea in the liver, and lost in the urine (Bach et al., 2005). It remains unclear if these observations are caused by changes in rumen microbial populations, dietary NPN, or by lack of synchrony in carbohydrate and N availability. In spite of the decrease in N retention as DDGS increased, retention at 14 % DDGS may be enough to support growth. At this level N retention is similar to the observed by Zanton and Heinrichs (2009a), at similar NI and in heifers with similar age and BW.

Nutrient excretion

Excretion parameters are presented in Table 7. Wet, dry, and water fecal outputs were higher for HF. This is comparable with previous reports where precision-fed heifers were offered diets with different F:C (Hill et al., 2007; Moody et al., 2007; Zanton and Heinrichs, 2009a; Lascano and Heinrichs, 2011). The increase of wet fecal output is proportional to the increase in F:C and consistent with the decrease in AD of DM and OM (Lascano and Heinrichs, 2011).

Also, as-fed intake and DMI g/kg of $BW^{0.75}$ were higher for HF, which may have contributed to greater fecal output. Higher fecal water excretion of HF could be in response to greater NDF intake and lower AD of these rations; Zanton and Heinrichs (2009a) determined that fecal water output was highly correlated to fecal NDF excretion. Urine output was statistically similar (P = 0.23) for HF vs. LF in spite of large numerical differences; LF output was 76% greater than HF. No difference in urine output is congruent with the observations of Moody et al. (2007), Zanton and Heinrichs (2009a) and Lascano et al. (2009); however, numerical increases for LF were observed: 2 kg/d in 12-mo-old heifers by Moody et al. (2007) and 3.4 kg/d by Zanton and Heinrichs (2009a). Other experiments reported significantly greater urine output at lower F:C (Hill et al., 2007; Lascano and Heinrichs, 2011). Greater urine excretion should be related to higher voluntary water intake since LF rations have greater DM%. Lascano and Heinrichs (2011) reported a 5.9 L/d numerical increase in voluntary water intake for LF, similar to the numerically (P = 0.57) greater 3 kg/d observed in the present study. Manure output was similar for HF vs. LF, which is comparable to the report by Lascano and Heinrichs (2011), who determined this is due to the inverse relationship between fecal water and urine output. Hill et al. (2007) observed greater manure excretion for LF while Lascano et al. (2009) for HF rations. Differences among the later trials are likely because urine output for LF in Hill et al. (2007) was 3-fold of HF while urine output was similar in Lascano et al. (2009).

Wet, dry, and water fecal outputs responded quadratically to DDGS level, with lower excretions at the 14% level. This was in response to AD of DM, OM, and fiber and was an inverse relationship, with higher AD at 14% DDGS. Leupp et al. (2009) observed that fecal output decreased linearly as DDGS level increased, and attributed it to a similar trend in OM intake. Linear interactions for wet, dry, and water fecal outputs, where differences due to DDGS

level were greater in HF rations, could be due to the higher fiber content of these diets. Urine and manure output were not affected by F:C.

Rumen digesta weight and volume

Weight and volume of rumen digesta (Table 5) were greater for HF and were not affected by DDGS level. Lower mass and volume for lower F:C is similar to previous results (Moody et al., 2007; Lascano and Heinrichs, 2009). Moody et al. (2007) attributed this observation to the greater DMI and reduced DM in situ digestibility of HF rations. In a review by Jung and Allen (1995) it was determined that rumen passage rate can be reduced by increasing forage fiber in the diet, because of its lower digestibility and the increase of ration particle size. In the present study, dietary peNDF of HF was higher (Table 9), which likely reduced passage rate, resulting in increased rumen digesta weight and volume.

Chewing activities and welfare

Results of chewing activities are shown in Table 8. Time spent eating tended to be longer for HF (2.5 vs. 1.9 h/d); this was expected as DMI was greater for HF, and similar observations have been previously reported. Kitts et al. (2011) altered DMI and F:C by adding straw (30% of DMI) to a limited amount of TMR (40 % forage) and observed that time spent eating increased from 1.2 h/d (heifers fed TMR only), to 3.4 and 4.8 h/d for heifers fed the straw on the side or in the TMR respectively. Also, addition of straw increased eating time of ad libitum-fed prepubescent heifers, from 3 h/d when fed a 69:31 F:C TMR, to 3.3 h/d when straw (20% of DM) was added to the TMR (Greter et al., 2008). Like in Kitts et al. (2011), feeding method also

affected time spent eating for heifers offered grass hay ad libitum with a fixed amount of concentrate at about 62:38 F:C, either separated, with grain top dressed, or as a TMR; heifers spent 2.3, 2.8 and 3.1 h/d eating respectively (DeVries and von Keyserlingk, 2009). Greater eating times have been reported for heifers with ad libitum access to hay and not supplemented with concentrates; Jaster and Murphy (1983) fed alfalfa hay as the only feed source to Holstein heifers and observed an average of 8.5 h/d eating. These data confirm the generally accepted theory that dairy cattle spend progressively more time eating as F:C or quantity of slowly digestible or indigestible feed intake increases (Beauchemin, 1991). Although feeding method was not part of the scope in the present study, it should be considered since, as shown in Kitts et al. (2011) and DeVries and von Keyserlingk (2009), it also affects time spent eating. Short eating times of heifers in the present study and limit fed heifers in Kitts et al. (2011) may be in part because animals fed to a restricted DMI increase their eating rate (g/min; Church, 1988). In Hoffman et al. (2007) when ad libitum intake of gravid Holstein heifers was compared to 80% of the ad libitum DMI, time spent eating decreased by 48%. This supports the previous statement that limit-fed heifers eat at a greater rate; limit-fed heifers used 52% of the time to eat 80% of the amount of feed compared to ad libitum-fed heifers. Likewise Kovacs et al. (1997) reported longer min/kg of DMI as intake level increased in steers. In the present study, heifers in the HF treatment spent 6 more min/kg of DMI eating, a numerical increase of 33% ($P = 0.12$). Increased eating time (min/kg of DMI) in response to higher F:C also was reported in late-lactating dairy cows (Zebeli et al., 2007). Thus eating time in limit-fed heifers is affected by F:C and as a possible behavioral response to feed restriction. Time spent eating and min/kg of DMI and NDFI were not affected by DDGS inclusion, and min/kg of NDFI was similar at the 2 levels of F:C.

Daily rumination time was similar for F:C; this differs from Zebeli et al. (2007), who observed that rumination time increased for rations higher in F:C fed to late-lactating dairy cows. It should be noted that in the present study a numerical difference of 38 min/d (P = 0.16), representing a 9% increase, was observed for HF. Interestingly, late-lactating cows in Zebeli et al. (2007) spent between 242 and 405 min/d ruminating, less than heifers in the present study that spent between 376 and 471 min/d ruminating. This is in spite of cows' DMI being twice the DMI of heifers. Consequently, ruminating min/kg of DMI and min/kg of NDFI of heifers were greater than those of late-lactating cows, suggesting that shorter eating times of limit-fed heifers are in part compensated for by lowering ruminating rate. In the present study heifers in LF spent 48 more min/kg of peNDFI ruminating, which suggests that heifers compensate for reduced peNDF in the ration by elongating the time they spend chewing each bolus. A similar compensatory behavior was observed by Metz (1975), who observed more rumination time per kg of intake as intake decreased in limit-fed non-pregnant, non-lactating cows fed long hay. However, when cows were fed hay wafers rumination time increased linearly as DMI increased. Conflicting results may be a consequence of forage particle length effect on chewing activities; greater particle length increased ruminating min/kg of DMI and time in late-lactating cows (Zebeli et al., 2007). Ruminating time per unit of DMI and NDFI was not affected by F:C in the present study, while Zebeli et al. (2007) observed higher ruminating min/kg of DMI for increased forage but similar min/kg of NDFI.

Despite NDF being similar across DDGS inclusion within F:C, daily ruminating time and ruminating min/kg of DMI, NDFI and peNDFI, linearly increased as DDGS level raised. When lactating cows were provided 10% DDG ruminating time and ruminating min/kg of DMI were not affected, there was a reduction in ruminating min/kg of NDFI, and time spent lying increased

by nearly 2 h/d (Penner et al., 2009). In the present study the increase in ruminating time and time per unit of DMI and NDFI as DDGS increased in the diet may be due to less fiber degradation by rumen microbes, as protozoa population (Table 6) decreased as DDGS increased. Digestion of forage fiber is faster in the presence of protozoa (Orpin, 1984); it was estimated that about a third of fiber digestion could be attributed to protozoa (Demeyer, 1981). Reduced microbial fiber degradation could be compensated for by more mechanical breakdown of fiber therefore ruminating effort is increased (De Boever et al., 1990).

Total chewing time (eating plus ruminating) tended to be longer for HF; this was expected since eating time had the same pattern. Yet, the tendency was only for a 15% change, while the tendency for eating time of HF to be longer was 35%, and ruminating time was numerically 9% longer for HF. Also, chewing min/kg of peNDFI tended to be shorter for HF. These together suggest that LF heifers somewhat compensated for less time spent eating and less peNDF in the ration by longer chewing time per unit of peNDFI. In contrast Zebeli et al. (2007) reported more chews per unit of DM and more min/kg of peNDFI with increased forage. Different results may be because animals in the Zebeli et al. (2007) study were lactating cows and had greater DMI; both level of intake (Sauvant et al., 1990) and body size (Bae et al., 1983) affect chewing activity. As in the present study, Zebeli et al. (2007) observed that chewing time decreased at lower F:C; this was also seen in steers fed different amounts and sources of concentrates (Sudweeks, 1977). As a result of longer ruminating time required as DDGS increased, total chewing was affected and showed a similar pattern.

Daily time spent licking of LF was numerically 30% more than that of HF. High standard errors may be the reason for no statistical significance in any parameters related to licking behavior. In this study licking as in self grooming or oral stereotypies was not differentiated, and

the times reported may be inflated since each observation was assumed to last for 5 min. Kononoff et al. (2002) compared the observational method used in this study with an automatic system and determined that the observational method overestimates daily eating and ruminating times by 3.6 and 10.3% respectively. Licking time was not compared in the Kononoff et al. (2002) study, but because licking is a more scattered activity and has shorter bouts, it might be overestimated to a greater degree by the observational method. Nevertheless the values are valid to discuss as frequency observations, though this limits the conclusions we can obtain from the data because a frequency increase does not necessarily mean an insult to the animal's welfare. Broom (1983) suggested that for conditions to be poor for an animal's welfare, stereotypies need to occur for 10% of its waking life, hence there is a need for time values to determine if stereotypies represent a threat to welfare. Also, stereotypies are part of the normal behavioral repertory, so more than their presence, their prolonged occurrence is what indicates if animal welfare is threatened (Broom, 1983). In theory, stereotypy occurrence would increase as F:C decreased by way of decreasing chewing activity. Lowered level of stereotypies as chewing time increased has been reported in lactating cows (Lindström and Redbo, 2000) and in limit-fed heifers (Redbo and Nordblad, 1997). Caution should be taken when addressing the effect of diet on stereotypic behavior of heifers housed in tie-stall facilities since tethering increases stereotypies (Redbo, 1992; Krohn, 1994). Also, the presence of feed in adjacent pens or out of reach intensified the stereotypic behavior of pigs (Rushen, 1985) and likely of heifers (Redbo, 1990). More research that quantifies the stereotypic behavior of limit-fed heifers fed various F:C is necessary to determine if this management strategy negatively affects animal welfare.

Kitts et al. (2011) suggested that limit-fed heifers are at greater risk of lameness because of long inactive standing time and risk of subacute ruminal acidosis. In their study heifers fed the

ration with lower F:C (more restricted) spent more time standing inactively; this observation is in agreement with the reported by Hoffman et al. (2007). However, heifers in the Kitts et al. (2011) study spent the same total time standing (inactive standing plus standing while eating). Although greater time standing does increase the risk of laminitis in lactating cows (Nocek, 1997; Cook et al., 2004), growing heifers may not be affected by it or may be affected to a lesser extent if total standing time is similar. Also, it can be speculated that out of reach feed in adjacent pens may be in part the reason why heifers stood inactively. The relationship between high concentrate diets, ruminal acidosis, and lameness is well established in lactating dairy cows (Nocek, 1997), but reports in growing heifers limit-fed such diets suggest that they are not at risk of acidosis. Lascano and Heinrichs (2009) evaluated 3 levels of F:C (either 80, 60 or 40% forage) fed at a restricted intake to growing dairy heifers and observed similar mean, minimum, and maximum ruminal pH. In the present study, mean rumen pH (Table 5) and the variation of pH during the day were not affected by F:C or DDGS level. The variation of pH during the day for LF and HF (Figure 1) confirms that heifers were not at risk of ruminal acidosis. Moody et al. (2007) observed lower pH for decreased F:C; however, levels did not represent a threat for rumen acidosis. Leupp et al. (2009) observed that pH increased linearly as DDGS increased, and suggested it was due to a decrease in starch concentration as DDGS increased. Rumen pH resilience of heifers limit-fed high concentrate diets may be due to the lower intake in comparison to ad libitum feeding systems (Lascano and Heinrichs, 2009).

Conclusions

Nutrient AD responded quadratically to DDGS level. Ammonia concentration and molar proportion of propionate increased; while molar concentration of acetate, acetate to propionate ratio, and rumen protozoa number decreased with increasing levels of DDGS. LF rations had greater DM and OM AD. Total chewing time increased by the addition of DDGS and higher F:C. DDGS influenced ruminating time with no effect on eating time, while F:C affected eating time. Heifers in LF partially compensated lower eating times by increasing ruminating time per unit of peNDF. Higher F:C increased rumen digesta weight and volume. Forage and DDGS levels did not affect rumen pH. DDGS can be successfully added to rations for precision-fed dairy heifers up to 14% inclusion before there is a decrease in AD, however nitrogen retention decreased linearly as DDGS was added to the basal ration. More research that quantifies the stereotypic behavior of limit-fed heifers fed various F:C is necessary to determine if this management strategy negatively affects animal welfare.

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Table 1. Ingredient and chemical composition of low- (LF) or high-forage (HF) dairy heifer diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

	LF				HF			
Item	0% DDGS	7% DDGS	14% DDGS	21% DDGS	0% DDGS	7% DDGS	14% DDGS	21% DDGS
Ingredients, % DM								
Grass hay	25.00	25.00	25.00	25.00	37.50	37.50	37.50	37.50
Corn silage	25.00	25.00	25.00	25.00	37.50	37.50	37.50	37.50
DDGS[1]	0.00	7.00	14.00	21.00	0.00	7.00	14.00	21.00
Ground corn	18.55	17.91	17.24	16.58	2.55	1.81	1.05	0.29
Soy hulls	8.00	8.00	8.00	8.00	.	.	.	.
Canola meal	20.45	13.84	7.26	0.67	19.45	12.95	6.48	.
Min/vit[2]	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Optigen[3]	.	0.25	0.50	0.75	.	0.24	0.48	0.72
Chemical composition								
DM, %	69.38	69.28	68.93	68.48	59.33	59.11	58.49	57.76
CP, % DM	15.31	14.59	15.02	15.12	13.96	13.90	14.15	14.19
Soluble P, % CP[4]	23.46	25.50	23.53	21.27	31.64	28.70	26.53	24.99
Soluble P, % CP[5]	23.46	28.57	29.49	30.15	31.64	31.77	32.56	34.02
RDP, % CP	43.59	43.78	45.15	45.32	54.47	55.28	52.63	54.77
ADF, %DM	26.17	26.82	26.02	25.23	29.33	28.88	28.93	28.23
NDF, % DM	39.94	41.81	42.12	41.69	46.27	46.47	47.17	47.60
Starch, % DM	23.12	21.45	21.86	22.53	18.66	17.89	17.79	17.44
EE, % DM	3.35	3.74	4.32	4.99	3.15	3.53	4.01	4.67
Ash, % DM	6.07	6.09	6.10	5.78	6.48	6.63	6.37	5.98
Ca, % DM	0.93	0.90	0.88	0.81	0.85	0.87	0.85	0.74
P, % DM	0.52	0.48	0.45	0.42	0.47	0.46	0.43	0.40
Mg, % DM	0.39	0.35	0.34	0.33	0.37	0.36	0.34	0.29
K, % DM	1.35	1.38	1.37	1.36	1.62	1.62	1.61	1.55
Na, % DM	0.18	0.23	0.24	0.26	0.20	0.21	0.22	0.21
Fe, ppm	227.38	204.28	221.01	213.93	203.56	205.33	204.10	193.65
Mn, ppm	107.13	93.30	94.95	88.70	101.19	102.11	97.86	85.66
Zn, ppm	106.25	86.87	84.02	89.57	66.88	71.36	68.14	59.40
Cu, ppm	29.50	30.36	28.74	27.13	27.00	27.78	28.53	20.77
TDN[6], % DM	70.84	70.30	71.15	72.59	67.36	67.73	68.49	69.54
NFC[7], % DM	37.38	34.73	35.03	35.63	31.41	30.85	30.93	30.94
ME[8], Mcal/kg	2.56	2.54	2.57	2.62	2.44	2.45	2.48	2.51

[1]Contained 88.1% DM, 31.4% NDF, 12.9% ADF, 29.1% CP, and 11.8% EE on DM basis, and 16 % SP of CP%.

[2]Contained 3.9% vitamin E, 0.59% vitamin ADE, 48.5% distillers corn with soluble vitamin D, 10.0% plain salt, 29.4% limestone, 4.6% magnesium oxide, 1.3% trace mineral premix, and 1.6% selenium premix.

[3]Optigen is a non-protein nitrogen source (256% CP, DM basis) from Alltech, Inc. (Lexington, Kentucky).

[4]Optigen soluble protein assumed to be 25% of CP.

[5]Optigen soluble protein assumed to be 95% of CP.

[6]Calculated.

[7]Calculated from ingredients as NFC = 100 – (NDF + CP + EE + ash).

[8]Estimated: ME = TDN × 0.04409 × 0.82.

Table 2. Nutrient intake of low- (L) or high-forage (H) dairy heifer diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, *P*-value				
		DDGS % in diet						DDGS		Interaction	
Item	F:C	0	7	14	21	SE	F:C	L	Q	L	Q
Intake, kg/d											
As fed	L	9.37	9.16	9.02	8.91	0.20	<0.01	<0.01	0.13	0.05	0.64
	H	11.33	10.94	10.81	10.60						
DM	L	6.50	6.35	6.21	6.10	0.13	0.52	<0.01	0.45	0.03	0.87
	H	6.72	6.47	6.32	6.12						
ADF	L	1.70	1.70	1.62	1.54	0.04	<0.01	<0.01	0.02	0.01	0.02
	H	1.97	1.87	1.83	1.73						
NDF	L	2.60	2.65	2.62	2.54	0.06	<0.01	<0.01	0.06	0.00	0.00
	H	3.11	3.01	2.98	2.91						
Ash	L	0.39	0.39	0.38	0.35	0.01	0.03	<0.01	<0.01	<0.01	0.16
	H	0.44	0.43	0.40	0.37						
TDN[1]	L	4.60	4.46	4.42	4.43	0.09	0.42	<0.01	0.02	0.11	0.38
	H	4.53	4.38	4.33	4.26						
Water, voluntary	L	31.29	24.89	30.49	24.26	4.80	0.57	0.64	0.96	0.39	0.98
	H	24.00	24.47	24.65	25.50						
Water, total	L	34.16	27.71	33.29	27.06	4.78	0.80	0.62	0.94	0.39	0.98
	H	28.61	28.94	29.14	29.98						
EE[2], g/d	L	217.65	237.32	268.44	304.72	5.30	0.14	<0.01	<0.01	<0.01	0.83
	H	211.77	228.65	253.53	285.87						
EE, g/d from grain	L	99.89	116.67	142.16	171.62	2.56	<0.01	<0.01	<0.01	0.07	0.81
	H	60.50	77.62	99.58	128.55						
ME[3], Mcal/d	L	16.48	15.93	15.68	14.70	0.39	0.29	<0.01	0.10	0.39	0.89
	H	15.74	15.34	15.04	14.27						
DM, g/kg $BW^{0.75}$	L	12.50	12.28	11.95	11.76	0.04	<0.01	<0.01	0.70	0.03	0.39
	H	12.75	12.47	12.15	11.81						
ME[3], g/kg $BW^{0.75}$	L	0.20	0.19	0.19	0.18	<0.01	0.01	<0.01	0.02	0.12	0.86
	H	0.19	0.18	0.18	0.17						

[1] Analyzed by Cumberland Valley Analytical Services Laboratory (Maungansville, MD).

[2] Analyzed EE from grain plus book values (NRC, 2001) EE from forages.

[3] Estimated: ME = TDN × 0.04409 × 0.82.

Table 3. Apparent nutrient digestibility of low- (L) or high-forage (H) dairy heifer diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, *P*-value				
		DDGS % in diet						DDG		Interaction	
Digestibility, %	F:C	0	7	14	21	SE	F:C	L	Q	L	Q
DM	L	66.92	67.16	68.16	64.74	0.96	0.02	0.13	0.01	0.32	0.31
	H	62.96	63.61	63.61	62.57						
OM	L	70.17	69.41	69.86	66.68	0.78	<0.01	0.01	0.02	0.03	0.85
	H	64.81	65.62	65.76	64.50						
ADF	L	41.20	48.39	50.84	43.86	2.16	0.15	0.06	<0.01	0.76	0.02
	H	39.90	42.05	43.52	41.97						
NDF	L	48.34	52.98	54.62	47.81	1.63	0.32	0.57	<0.01	0.59	0.04
	H	47.34	49.39	50.71	48.22						

Table 4. Nitrogen intake, apparent digestibility, and dynamics of dairy heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, *P*-value				
		DDGS % in diet						DDGS		Interaction	
Item	F:C	0	7	14	21	SE	F:C	L	Q	L	Q
Intake											
g/d	L	159.18	148.23	149.32	147.57	2.98	0.13	<0.01	<0.01	0.90	0.02
	H	150.21	143.84	143.16	138.94						
g/kg $BW^{0.75}$	L	1.91	1.79	1.79	1.78	0.01	<0.01	<0.01	<0.01	0.05	<0.01
	H	1.78	1.73	1.72	1.67						
Digestibility, %	L	64.66	63.26	67.47	64.46	1.27	0.22	0.13	0.91	0.50	0.32
	H	63.19	62.21	64.42	65.47						
Fecal N, g/d	L	56.25	54.49	48.66	52.55	2.12	0.60	<0.01	0.57	0.51	0.20
	H	55.38	54.51	50.96	47.90						
Urinary N, g/d	L	61.10	63.67	64.11	67.28	3.08	0.08	0.02	0.46	0.79	0.56
	H	54.80	57.09	55.82	63.07						
Retained N											
g/d	L	41.83	30.07	36.56	27.74	4.36	0.98	0.01	0.81	0.86	0.70
	H	40.03	32.24	36.38	27.97						
% of intake	L	26.23	20.35	24.41	18.88	2.81	0.68	0.03	0.89	0.94	0.81
	H	26.67	22.60	25.43	20.10						
% of digested	L	40.08	31.50	36.18	29.25	3.91	0.54	<0.01	0.92	0.87	0.64
	H	42.05	35.70	39.39	30.49						

Table 5. Rumen fermentation parameters and weight of dairy heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, *P*-value				
		DDGS % in diet						DDGS		Interaction	
Item	F:C	0	7	14	21	SE	F:C	L	Q	L	Q
NH_3, mg/dl	L	6.25	5.72	6.54	7.41	0.88	0.07	0.08	0.49	0.79	0.69
	H	6.67	7.97	7.20	8.87						
VFA, mM	L	103.61	101.87	98.29	93.49	4.78	0.80	0.02	0.29	0.40	0.69
	H	101.25	104.77	100.16	97.08						
VFA, molar % of total VFA											
Acetate	L	64.94	64.45	64.02	62.59	0.68	0.07	<0.01	0.14	0.18	0.99
	H	65.94	66.73	65.33	65.17						
Propionate	L	18.36	19.01	19.28	21.15	0.36	0.29	<0.01	0.01	0.02	0.45
	H	19.20	18.37	18.87	20.00						
Butyrate	L	12.38	12.70	12.51	11.80	0.53	0.29	0.96	0.13	0.14	0.86
	H	11.34	11.36	12.47	11.66						
Valerate	L	1.73	1.56	2.09	1.79	0.22	0.10	0.69	0.71	0.26	0.81
	H	1.36	1.30	1.33	1.24						
Isobutyrate	L	1.09	1.08	0.99	1.16	0.05	0.01	0.45	0.29	0.16	0.16
	H	0.97	0.93	0.91	0.86						
Isovalerate	L	1.50	1.20	1.11	1.51	0.12	0.16	0.37	0.08	0.45	0.02
	H	1.19	1.30	1.09	1.07						
Branched-chain	L	4.32	3.84	4.19	4.46	0.26	0.02	0.71	0.35	0.13	0.13
	H	3.52	3.54	3.33	3.16						
Acetate:propionate	L	3.54	3.40	3.33	2.96	0.08	0.12	<0.01	0.01	0.03	0.42
	H	3.44	3.64	3.47	3.27						
pH	L	6.42	6.48	6.40	6.56	0.08	0.70	0.75	0.55	0.18	0.56
	H	6.47	6.41	6.45	6.39						
Rumen contents[1]											
Weight, kg	L	37.36	36.48	37.86	38.78	2.40	0.03	0.70	0.45	0.04	0.66
	H	47.81	47.21	45.69	45.56						
Volume, L	L	42.60	40.21	40.96	42.33	2.75	0.03	0.59	0.66	0.60	0.13
	H	51.75	51.94	52.08	50.19						
Heifer weight, kg	L	375	371	374	375	10.88	0.87	0.09	0.25	0.03	0.79
	H	381	377	374	373						
Empty BW[2], kg	L	337	335	336	336	9.27	0.64	0.12	0.38	0.18	0.93
	H	334	330	328	327						

[1]Determined by whole rumen contents evacuation.

[2]Determined by subtracting rumen content weight from heifer BW before rumen evacuation.

Table 6. Urinary purine derivatives (PD) excretion, estimated bacterial CP flow, and rumen protozoa population of dairy heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, *P*-value				
		DDGS % in diet						DDGS		Interaction	
Item	F:C	0	7	14	21	SE	F:C	L	Q	L	Q
Allantoin, mmol	L	96.54	77.59	88.78	83.76	12.28	0.42	0.89	0.73	0.34	0.36
	H	67.56	80.39	70.93	77.44						
Uric acid, mmol	L	9.96	8.75	8.58	7.92	1.76	0.21	0.65	0.99	0.21	0.72
	H	5.24	6.19	5.96	6.32						
PD, mmol	L	106.50	86.34	97.36	91.68	13.84	0.39	0.85	0.75	0.30	0.38
	H	72.80	86.58	76.89	83.76						
Microbial CP[1], g/d	L	398.18	291.31	349.26	319.13	75.33	0.39	0.87	0.77	0.29	0.38
	H	215.44	291.92	239.71	277.04						
Allantoin, % of PD	L	90.85	90.04	91.22	91.61	0.93	0.09	0.82	0.52	0.30	0.73
	H	92.86	92.89	92.01	92.39						
Protozoa[2], $\times 10^4$/mL	L	27.32	28.80	24.29	11.41	4.46	0.68	<0.01	<0.01	0.25	0.96
	H	21.51	25.88	22.84	12.48						

[1] Estimated according to the methods and equations of Chen and Gomes (1992).

[2] Actual protozoa counts were $\log_{10}$-transformed for the statistical analysis.

Table 7. Excretion parameters of dairy heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, *P*-value			
		DDGS % in diet					DDGS		Interaction	
Item	F:C	0	7	14	21	SE	F:C	L	Q	L	Q
Wet feces, kg/d	L	11.66	11.07	11.01	11.82	0.29	<0.01	0.02	0.01	<0.01	0.3
	H	15.03	14.49	13.67	13.76						
Dry feces, kg/d	L	2.15	2.09	1.98	2.16	0.06	<0.01	0.02	0.01	0.06	0.42
	H	2.49	2.36	2.30	2.29						
Fecal water, kg/d	L	9.51	8.98	9.03	9.66	0.27	<0.01	0.02	0.02	<0.01	0.3
	H	12.54	12.14	11.37	11.46						
Urine, kg/d	L	15.44	10.04	13.35	9.58	3.73	0.23	0.51	0.97	0.38	0.72
	H	5.97	7.82	6.59	7.10						
Manure kg/d	L	27.10	21.11	24.36	21.40	3.74	0.57	0.37	0.78	0.54	0.65
	H	21.01	22.31	20.26	20.85						

Table 8. Chewing activities of dairy heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21% dry distillers grains with solubles (DDGS)

							Contrast, *P*-value				
		DDGS % in diet						DDGS		Interaction	
Item	F:C	0	7	14	21	SE	F:C	L	Q	L	Q
Time, min/d											
Eating	L	114	107	123	104	15	0.09	0.93	0.71	0.64	0.44
	H	149	153	147	154						
Ruminating	L	389	376	424	429	21	0.16	0.03	0.03	0.25	0.16
	H	454	421	422	471						
Licking	L	259	288	296	261	53	0.39	0.95	0.16	0.91	0.82
	H	203	224	226	201						
Chewing	L	503	483	547	533	33	0.10	0.09	0.11	0.46	0.15
	H	603	574	569	626						
Idle	L	649	653	581	626	42	0.76	0.29	0.95	0.72	0.38
	H	617	618	626	591						
Min/ kg DMI											
Eating	L	17.57	16.74	19.76	16.99	2.52	0.12	0.23	0.67	0.37	0.51
	H	22.25	23.85	23.27	25.30						
Ruminating	L	59.80	59.11	68.37	70.16	3.23	0.21	< 0.01	0.05	0.53	0.17
	H	67.49	65.44	66.74	77.22						
Licking	L	39.61	45.89	47.58	43.28	8.47	0.36	0.43	0.17	0.91	0.79
	H	30.22	34.81	35.71	33.07						
Chewing	L	77.37	75.84	88.13	87.15	5.30	0.15	< 0.01	0.15	0.90	0.18
	H	89.74	89.29	90.01	102.52						
Idle	L	100.43	102.54	94.27	102.86	6.92	0.61	0.59	1.00	0.55	0.35
	H	91.80	95.60	99.07	96.41						
Min/ kg NDFI[1]											
Eating	L	44.18	39.92	46.93	40.77	5.80	0.35	0.57	0.88	0.36	0.74
	H	48.12	51.32	49.30	53.24						
Ruminating	L	149.91	141.49	162.51	168.39	7.29	0.36	< 0.01	0.02	0.45	0.46
	H	146.03	140.86	141.59	162.44						
Licking	L	98.54	110.61	112.84	103.88	20.39	0.23	0.64	0.22	0.93	0.85
	H	65.69	75.05	75.88	69.61						
Chewing	L	194.09	181.41	209.44	209.16	12.03	0.98	0.01	0.06	0.82	0.46
	H	194.14	192.18	190.89	215.67						
Idle	L	251.88	244.73	224.45	246.96	16.30	0.09	0.80	0.64	0.45	0.16
	H	198.19	206.14	209.87	202.69						
Min/kg peNDFI[2]											
Eating	L	70.12	65.82	77.68	66.98	8.93	0.99	0.32	0.65	0.41	0.53
	H	66.20	71.02	69.11	74.95						
Ruminating	L	238.61	232.42	268.76	276.54	11.38	<0.01	<0.01	0.06	0.27	0.37
	H	200.81	194.88	198.21	228.81						
Licking	L	158.08	180.45	187.05	170.60	32.91	0.13	0.49	0.20	0.86	0.71
	H	89.91	103.67	106.05	97.98						
Chewing	L	308.73	298.25	346.44	343.53	18.59	0.08	<0.01	0.14	0.54	0.32
	H	267.01	265.90	267.32	303.77						
Idle	L	400.75	403.21	370.57	405.45	25.72	0.01	0.78	0.79	0.53	0.27
	H	273.14	284.70	294.23	285.66						

[1] NDF intake.

[2] Physically effective NDF intake; peNDF = proportion of particles > 1.18 mm determined by ASABE particle separator multiplied by NDF concentration of feed.

Table 9. Particle size of forage mix[1] and low- (LF) or high-forage (HF) dairy heifer diets.

		Diet	
	Forage mix	HF	LF
Xgm[2]	7.28	5.71	4.21
Sgm[3]	3.64	3.15	2.70
peNDF[4]	43.31	33.66	25.33

[1]Forage mix was 50% corn silage and 50% grass hay.

[2] Geometric mean length as determined by ASABE (2007).

[3]Standard deviation as determined by ASABE (2007).

[4] Physically effective NDF; peNDF = proportion of particles > 1.18 mm determined by ASABE particle separator multiplied by NDF concentration.

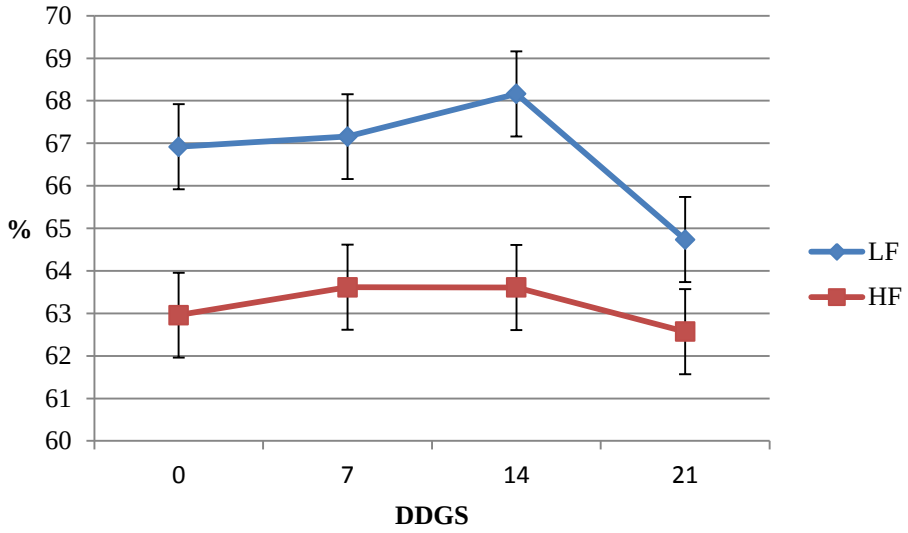

Figure 1. Dry matter apparent digestibility in heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21 % dry distillers grains with solubles (DDGS).

*$P < 0.05$ quadratic effect for DDGS level.
**$P < 0.05$ forage concentration main effect.

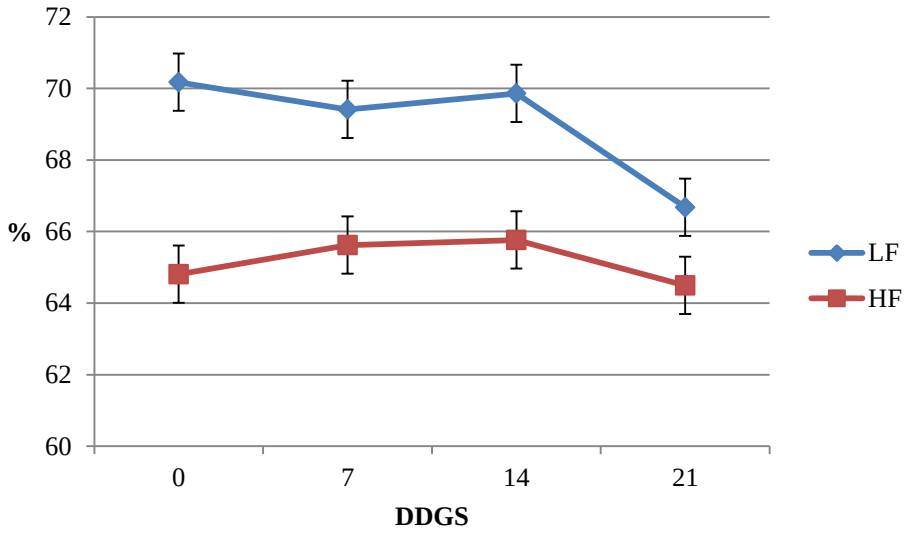

Figure 2. Organic matter apparent digestibility in heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21 % dry distillers grains with solubles (DDGS).

*$P < 0.05$ quadratic effect for DDGS level.
**$P < 0.01$ forage concentration main effect.
***$P < 0.05$ DDGS × forage linear interaction.

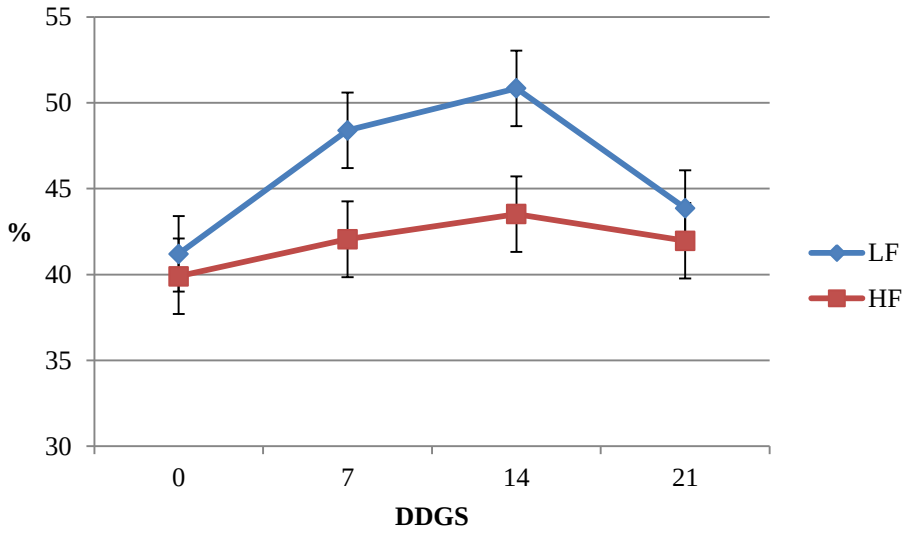

Figure 3. ADF apparent digestibility in heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21 % dry distillers grains with solubles (DDGS).

**P* < 0.01 quadratic effect for DDGS level.
***P* > 0.05 forage concentration main effect.
****P* < 0.05 DDGS × forage quadratic interaction.

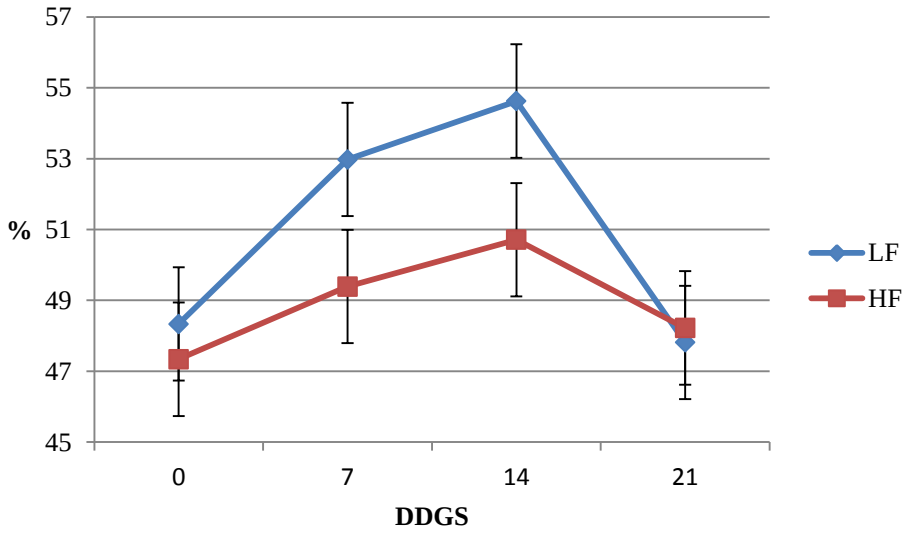

Figure 4. NDF apparent digestibility in heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21 % dry distillers grains with solubles (DDGS).

*$P < 0.01$ quadratic effect for DDGS level.
**$P > 0.05$ forage concentration main effect.
***$P < 0.05$ DDGS × forage quadratic interaction.

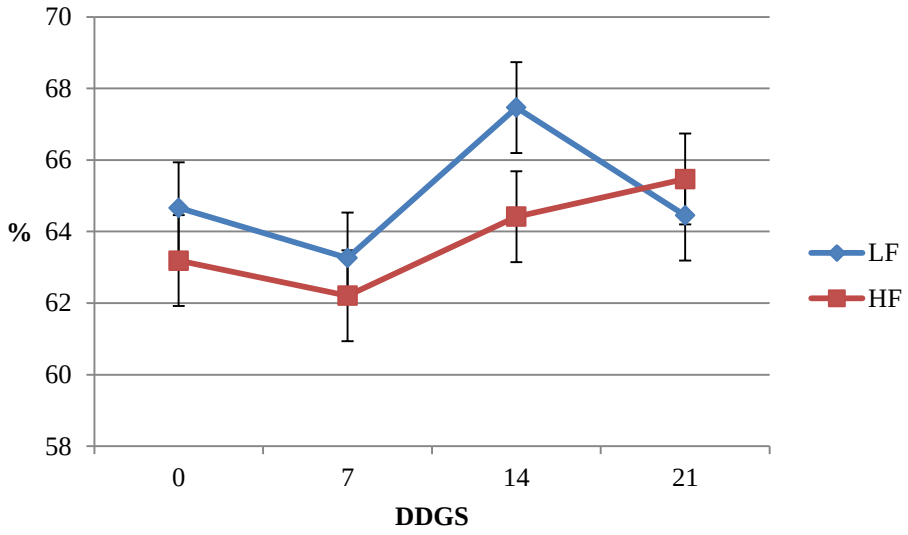

Figure 5. Nitrogen apparent digestibility in heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21 % dry distillers grains with solubles (DDGS).

*P > 0.05 effect for DDGS level.
**P > 0.05 forage concentration main effect.

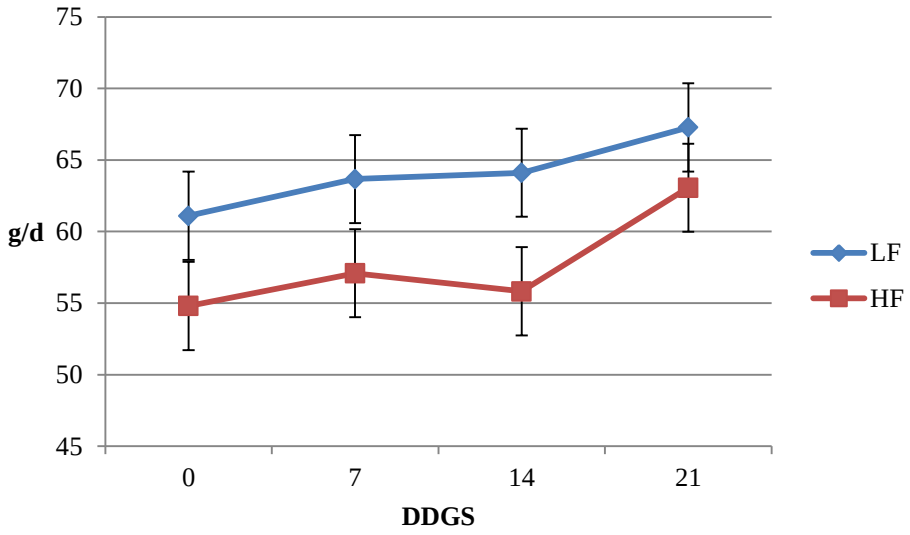

Figure 6. Nitrogen excreted in urine of heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21 % dry distillers grains with solubles (DDGS).

*$P < 0.05$ linear effect for DDGS level.
**$P < 0.1$ forage concentration main effect.

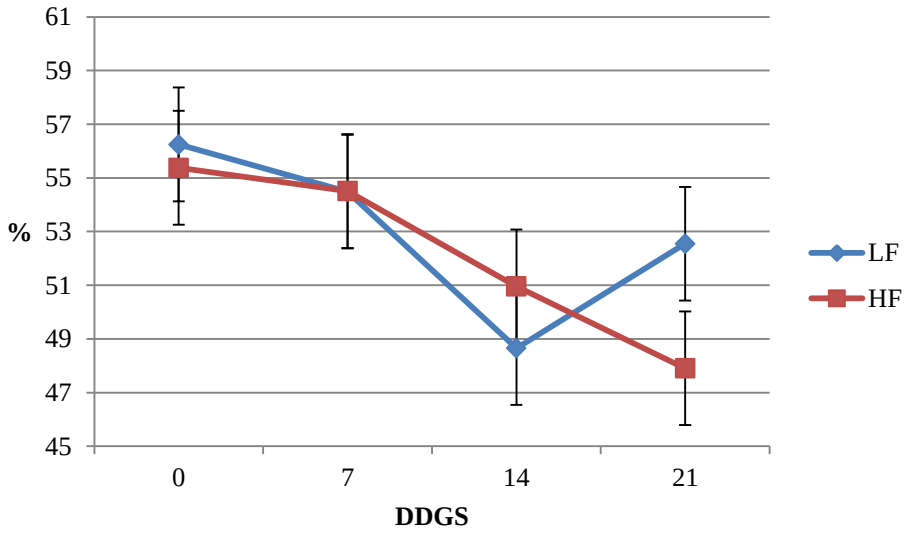

Figure 7. Nitrogen excreted in feces of heifers fed low- (L) or high-forage (H) diets containing 0, 7, 14, or 21 % dry distillers grains with solubles (DDGS).

*$P < 0.01$ linear effect for DDGS level.
**$P > 0.1$ forage concentration main effect.

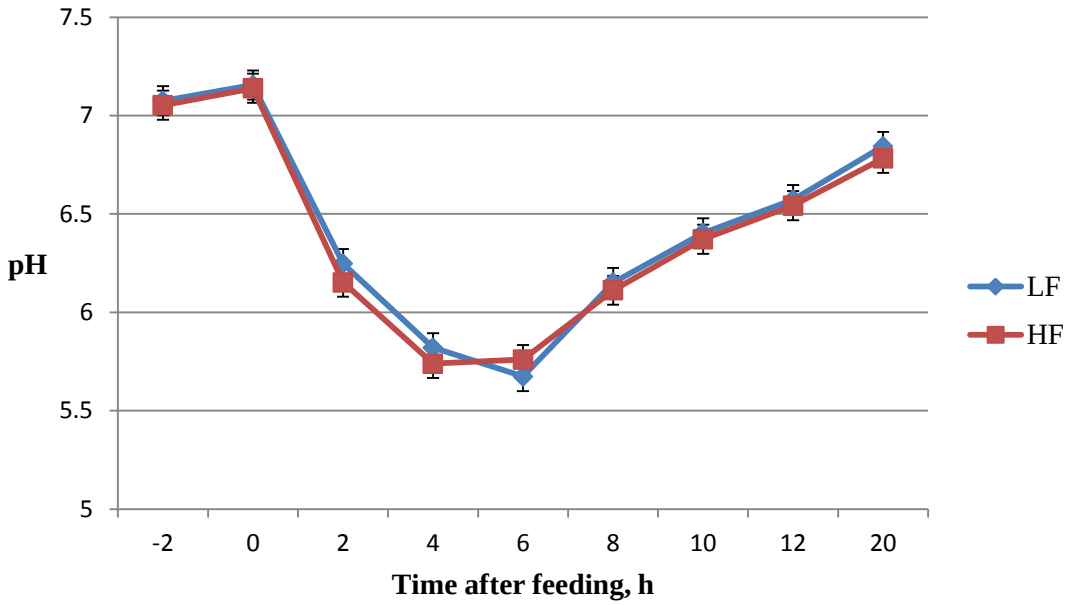

Figure 8. Mean rumen pH variation after feeding in heifers fed low- (LF) or high-forage (HF).

* $P > 0.7$ for forage × time interaction

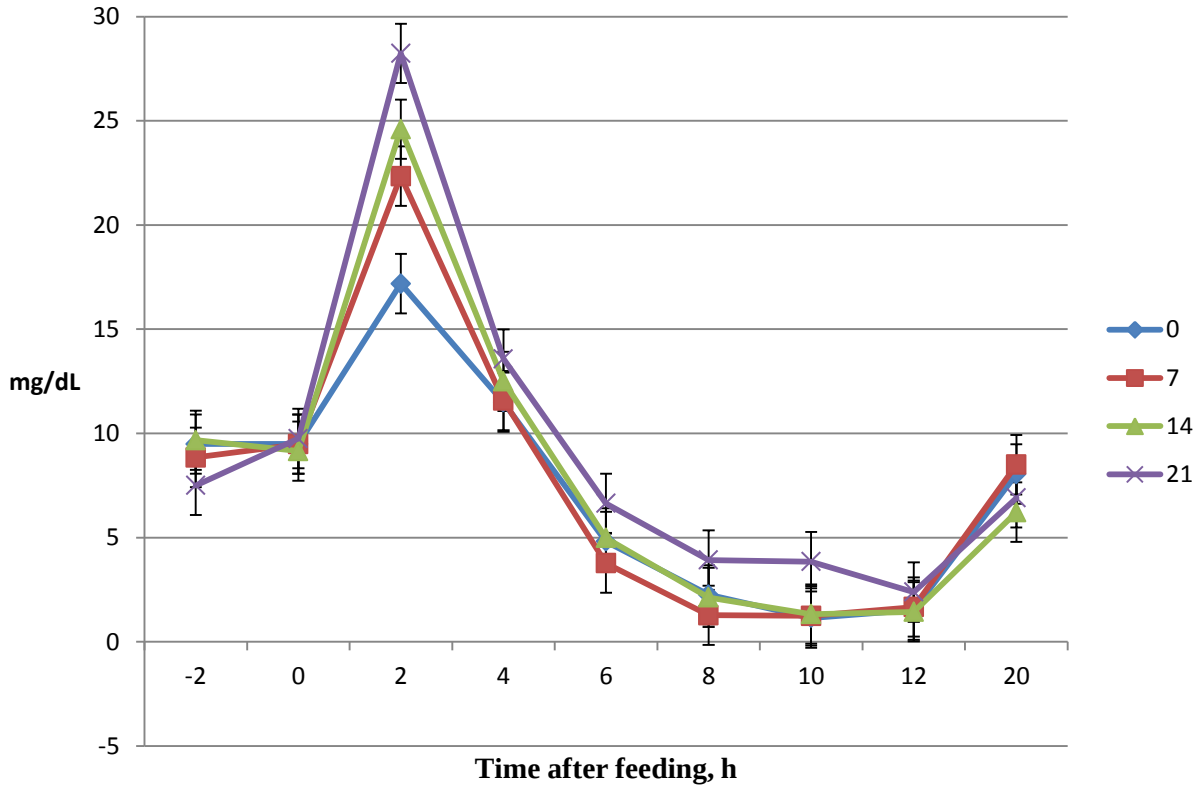

Figure 9. Mean rumen ammonia variation after feeding in heifers fed rations containing 0, 7, 14 or 21% corn dry distillers grains with solubles (DDGS).

* $P < 0.05$ for DDGS × time interaction

Appendix A

Abbreviations List

A:P = acetate to propionate ratio

AA = amino acid

AD = apparent digestibility

ADF = acid detergent fiber

ADG = average daily gain

AFC = age at first calving

BCS = body condition scoring

BW = body weight

CP = crude protein

CS = corn silage

DDG = corn dry distillers grains

DDGS = corn dry distillers grains with solubles

DG = distillers grains

DM = dry matter

DMI = dry matter intake

DRC = dry rolled corn

EE = ether extract

F:C = forage to concentrate ratio

HF = high forage ration

LF = low forage ration

ME = metabolizable energy

N = nitrogen

NAD = nitrogen apparent digestibility

NDF = neutral detergent fiber

NDFI = neutral detergent fiber intake

NFC = non fiber carbohydrates

NH_3 = ammonia

NI = nitrogen intake

NPN = non protein nitrogen

OM = organic matter

peNDFI = physical effective neutral detergent fiber intake

RUP = rumen undegradable protein

SBM = soy bean meal

TMR = total mixed ration

VFA = volatile fatty acids

WDG = corn wet distillers grains

WDGS = corn wet distillers grains with solubles

Appendix B

Forage Analysis

Item	Forage Mix[1]
DM %	49.08
CP % DM	7.77
Soluble P % CP	41.90
RDP % CP	75.95
Starch % DM	21.19
ADF %DM	31.41
NDF % DM	52.62
EE % DM	2.60
ASH % DM	4.83
Ca %DM	0.33
P % DM	0.26
Mg % DM	0.16
K % DM	1.65
Na % DM	0.02
Fe PPM	171.75
Mn PPM	56.25
Zn PPM	23.50
Cu PPM	6.00
TDN % DM	66.18
NFC % DM	32.15

[1]Forage mix was 50% corn silage and 50% grass hay, DM basis.