Evaluation of growth performance, nutrient utilization, metabolic profile and onset of puberty in dairy heifers fed reduced-fat distillers grains in replacement of forage in limit-fed rations

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ABSTRACT/SUMMARY

The objective of this study was to determine the maximum inclusion rate of reduced-fat distillers dried grains (**RFDDGS**) in limit-fed dairy heifer rations and to determine the effects on growth performance, metabolic profile, and onset of puberty. A 16-week randomized complete block design study was conducted using 48 Holstein heifers (199 ± 2 days of age) to evaluate effects of diet on dry matter intake (DMI), average daily gain (ADG), growth performance, rumen fermentation, and nutrient digestibility. Treatments were 1) 30% RFDDGS (**30DG**), 2) 40% RFDDGS (**40DG**), and 3) 50% RFDDGS (**50DG**) with the remainder of the diet consisting of grass hay and 1.5% mineral mix. Heifers were individually limit-fed using Calan gates at 2.65, 2.50, and 2.35% of body weight (BW) on a dry matter (DM) basis for 30DG, 40DG, and 50DG, respectively. Body weights, frame measurements and body condition score (BCS) were recorded every 2 weeks. Jugular blood samples were collected during weeks 0, 4, 8, 12, and 16 for metabolite and metabolic hormone analysis. Every 3 or 4 days coccygeal vein blood samples were taken for progesterone analysis to estimate puberty onset. Rumen fluid was collected via esophageal tubing during wk 12 and 16 for ammonia N and volatile fatty acid (VFA) analysis. Total tract digestibility of nutrients was evaluated during wk 16 using fecal grab sampling. Heifer DM intake linearly decreased with increasing concentrations of RFDDGS. Body weight and ADG was similar among treatments, while gain: feed ratio linearly increased across treatments. Frame growth was comparable among treatments and any statistical differences were numerically small. Blood metabolites glucose, insulin, leptin, and triglycerides were similar among treatments, while there was a quadratic effect for cholesterol and plasma urea nitrogen, and a quadratic tendency for IGF-1. There were also shifts in blood FA profile. Metabolic profile results demonstrated that fat metabolism was slightly shifted among treatments, but energy status was maintained in agreement with growth performance results. Average age and weight at puberty was similar among treatments. As the dietary concentrations of RFDDGS increased, pH linearly decreased across treatments and ammonia N linearly increased. Acetate proportion and acetate: propionate linearly decreased as RFDDGS increased, while propionate linearly increased, which partially helps support the increase in feed efficiency. Increasing dietary concentrations of RFDDGS linearly increased total tract digestibility of DM, organic matter, and crude protein (CP). Results demonstrated that replacing forage with RFDDGS in limit-fed rations can maintain heifer growth performance and energy status without having detrimental effects on age or BW at puberty. Increasing the utilization of DDGS is mutually beneficial to the corn ethanol industry and the dairy industry. It benefits corn growers by increasing the market for the corn ethanol co-products. Estimates based on heifer intakes showed that increasing the proportion of RFDDGS fed in the ration decreased heifer feeding costs. Therefore, utilizing more RFDDGS gives dairy producers an option to decrease overall rearing costs while maintaining growth performance and increasing feed efficiency.

INTRODUCTION

Previous research has demonstrated that feeding dried distillers grains with solubles (**DDGS**) has improved feed efficiency in ruminants (Anderson et al., 2006; Klopfenstein et al., 2008). The increased concentrations of fermentable fiber, and rumen undegradable protein found in DDGS compared to other feed sources such as corn and soybean meal are thought to be the cause of the improvement in animal production. Feeding DDGS has been well researched in beef heifers; however, there is limited research on feeding DDGS to dairy heifers. Dried distillers grains have been shown to be a replacement for corn and soybean meal in dairy heifer diets without causing changes in average daily gain (ADG) or negative effects on long-term performance (Anderson et al., 2015a).

Feeding DDGS to dairy heifers has been limited to high forage diets (Anderson et al., 2009; Anderson et al., 2015a). No research that we are aware of has examined the effects of replacing energy and protein from forage with energy and protein from DDGS in dairy heifer rations. In other words, research has not been conducted where DDGS has been the main concentrate in limit-fed dairy heifer rations. The high fat content of traditional DDGS, which is typically 10-15% ether extract, made this feeding strategy difficult. However, the development and availability of reduced-fat DDGS (**RFDDGS**), that has some of the fat removed through centrifugation, should allow it to be incorporated into the diet at much greater proportions.

Very limited research has been conducted feeding RFDDGS to dairy heifers. Schroer et al. (2014) fed heifers that were approximately 5 months of age one of three diets: a control, DDGS, or RFDDGS diet. Heifers were fed for 12 weeks intake, feed efficiency, and growth was measured. However, this study only incorporated RFDDGS at 20% of the diet DM. Heifers fed the RFDDGS had similar ADG, feed efficiency, hip height, and withers height as heifers fed the control diet and DDGS. This demonstrated that RFDDGS did not negatively affect heifer growth and that RFDDGS is a viable feed source for dairy heifers (Schroer et al, 2014). Anderson et al., (2015a) limit-fed dairy heifers with diets of approximately 22% low-fat DDGS with ground corn compared to 33% full-fat DDGS or a control diet, with equal forage concentrations for six months and also found similar growth performance among treatments.

Age and size are the two frequently measured factors that play a role in puberty attainment. Dairy heifers usually reach puberty between 9 and 11 months of age at an average BW of 250 to 280 kg (Sejrsen and Purup, 1997). In beef heifers, an increase in ADG can influence the age and weight at which heifers attain puberty with heifers, with an increased ADG resulting in heifers being heavier at puberty (Short and Bellows, 1971). This increase in ADG may cause an increase in adipose deposition and an increase in leptin concentrations. Low ADG have been linked to decreased reproductive performances with decreased percentage

bred, reduced pregnancies among animals bred, and higher pregnancy loss (Short and Bellows, 1971).

In dairy heifers increased prepubertal ADG has shown to affect milk production. Several researchers have shown that an increased ADG during the prepubertal period affected the development of parenchymal tissue in the mammary gland, resulting in decreased milk production (Hoffman and Funk, 1992; Sejrsen and Purup, 1997). This may be partially explained by IGF-1 receptors in the mammary tissues being less responsive when high energy diets are fed. This has been shown by reduced circulating growth hormones concentrations possibly as the result from negative feedback and an increase in circulating IGF-1 (Sejrsen and Purup, 1997).

We hypothesized that by using limit-feeding increasing the dietary concentration of RFDDGS would maintain heifer growth performance. However, we expected changes in metabolic hormones and profile specifically related to energy metabolism. We also expected some shift rumen fermentation as RFDDGS replaced forage in the diets. We also hypothesized that gain to feed and nutrient utilization would increase with increasing concentrations of RFDDGS.

B. PROJECT OBJECTIVES AND GOALS

1. The objective of this study was determine maximum or upper limits of dietary inclusion rates of RFDDGS in limit-fed rations by evaluating effects on growth, rumen fermentation, and total tract digestibility of nutrients.

2. A second objective was to determine the effect of feeding increased amounts of RFDDGS on the attainment of puberty and metabolic profile and hormone status of growing dairy heifers.

3. Another objective of the research is to determine how energy and protein from RFDDGS is utilized in replacement of grass hay in limit-fed growing dairy heifer rations.

All goals and objectives were successfully met for this project.

C. DESCRIPTION OF WORK PERFORMED (MATERIALS AND METHODS)

All procedures and animal use was approved prior to the start of the feeding study by the South Dakota Institutional Animal Care and Use Committee.

Experimental Design

Forty-eight Holstein heifers (199 ± 2 days of age) were used in a randomized complete block design with three treatment diets. Heifers were blocked in groups of three based on birth date and BW. Heifers were randomly assigned to treatment after assignment to block. Heifers were added to the study based on farm calving rates and were started on trial in multiples of six with the target age of 7 months at the beginning of the experimental feeding period. Heifers were acclimated to the research barns and feeding system for approximately two weeks followed by an experimental feeding period of 16 weeks.

Treatment diets (Table 1) were: 1) high forage with 30% of diet as RFDDGS (**30DG**), 2) moderate forage with 40% of diet as RFDDGS (**40DG**), and 3) low forage with 50% of diet as RFDDGS (**50DG**) on DM basis. The forage portions of the diets consisted of grass hay. Diets were formulated using the NRC (2001) to provide for 0.8 kg/d ADG when fed to a 250 kg BW Holstein heifer. The 250 kg BW was a rough pre-estimated average BW for heifers during the study based on age and herd data. The amount of feed offered was determined as a percentage of BW and decreased with increasing concentrations of RFDDGS in order to have similar intakes of energy across treatments. Diets were fed at 2.65, 2.50, and 2.35% of BW for 30DG, 40DG, and 50DG, respectively (DM basis).

In order to avoid variation in production within plant and over time, RFDDGS was purchased in two batches, one at the beginning of the experiment, and second batch half way through the study and stored at the South Dakota State University feed mill. Hay was purchased in two batches and effort was made to match the nutrient composition between batches.

Animal Care and Feeding

This feeding study was conducted at the South Dakota State University Dairy Research and Training Facility (SDSU DRTF; Brookings, SD). The study was completed from September 2013 through September 2014 because of the staggered start dates for each group of heifers and pen availability. Heifers were observed daily for health problems and treated according to routine management practices at the DRTF.

Heifers were housed in pens in groups of 6 heifers. Each pen had an inside roofed area and an outside small dirt exercise lot. The inside areas of the pens were a bedded pack, and were only bedded with straw once every 2 weeks. Each pen was provided with water ad libitum. Heifers were fed once daily at 0830 hours using the Calan gate feeding system (American Calan Inc., Northwood, NH) so that individual intakes could be measured. As previously mentioned, diets were limit-fed to 2.65, 2.50, and 2.35% of BW for 30DG, 40DG, and 50DG, respectively. Diets were adjusted every 2 weeks based on heifer BW and measurements, as well as DM analysis of feedstuffs. Diet components (RFDDGS, grass hay) were individually weighed into a large tub for each heifer and then hand mixed before being delivered to the Calan box. The mineral mix was individually weighed for each heifer and mixed with the RFDDGS before mixing with the grass hay. Because heifers were limit-fed and were expected to

consume all feed, particle sorting was a minor concern. Bales of hay were coarsely pre-ground with a vertical tub grinder to ease hand mixing. In order to determine individual daily intakes, any orts were weighed and recorded every morning before feeding. Samples of RFDDGS and grass hay were taken each week and stored at -20°C until analysis.

Animal Measurements and Sampling

Body growth measurements including BW, withers and hip heights, heart and paunch girth, body length, and hip width were taken on 2 consecutive days approximately 4 hours post-feeding at the beginning of the study and then every 2 weeks thereafter for the remainder of the study. Body length was measured from the top point of the withers to the end of the ischium (Hoffman, 1997). Body condition score (BCS) was assessed at the start of the experiment and then every 2 weeks thereafter for the remainder of the study by 3 independent observers based on the scale described by Wildman et al. (1982) with 1=emaciated and 5=obese.

Blood samples were taken on two consecutive days during weeks 0, 4, 8, 12, and 16 of the feeding study. Blood samples were taken approximately 4 hours post-feeding (1230 h) via venipuncture of the jugular vein into vacutainer tuber (Becton, Dickinson, and Company, Franklin Lakes, NJ) containing sodium fluoride (NaFl) and potassium oxalate for glucose analysis (Cat. # 367925) or potassium ethylene diamine tetra-acetic acid (K_2EDTA) for all other analyses (Cat. #366643). Following blood collection, samples were immediately placed on ice and brought into the laboratory for processing within 3 hours. Blood collection tubes were centrifuged at 1000 × g for 20 minutes at 4°C (Centrifuge CR412 Jouan, Inc., Winchester, VA). Plasma (K_2EDTA tubes) or serum (NaFl and K-oxalate tubes) was then transferred to polystyrene tubes using a plastic transfer pipette, and frozen at -20°C until further processing and analysis. When samples were analyzed, plasma or serum from the two consecutive days during each of the blood sampling weeks (0, 4, 8, 12, and 16) were both analyzed and then averaged with the exception of insulin in which plasma from the first day during each of the sampling weeks was analyzed.

Rumen fluid was sampled from each heifer on 2 consecutive days during weeks 12 and 16 approximately 4 hours post-feeding via esophageal tubing. After discarding the first 200 ml of fluid to minimize saliva contamination, approximately 50 mL of rumen fluid was collected. Samples were immediately measured for pH using a portable handheld pH meter (Waterproof pH Tester 30, Oakton Instruments, Vernon Hills, IL) and 2 aliquots (10mL) were acidified with either 200 µL of 50% (volume/volume) sulfuric acid or 2 mL of 25% (weight/volume) metaphosphoric acid and stored at -20°C until later analyses of ammonia N (NH_3-N) and volatile fatty acid (VFA) analysis, respectively.

Fecal samples for analysis of total tract digestibility were collected during week 16 of the feeding period. Acid detergent insoluble ash (ADIA) was used as an internal digestibility

marker. Over 3 consecutive days in week 16, orts and fecal grab samples were collected. Fecal sampling time points were scheduled so that in the end the samples represented every 3 hours over a 24 hour period of time relative to time of feeding. Orts and fecal samples were stored at -20°C until processing and analysis.

Laboratory Analysis

Feed samples were dried for 24 hours at 105°C every 2 weeks for DM analysis in order to adjust dietary ingredient inclusion rates and determine DMI. Samples of RFDDGS and grass hay were collected once weekly and frozen at -20°C until analysis. Samples of RFDDGS and grass hay were thawed and samples from 4 consecutive weeks were composited on an as-fed basis by weight. Composite samples were dried in duplicate for 48 hours at 55°C in Despatch oven (Style V-23, Despatch Oven Co. Minneapolis, MN), ground to 4 mm particle size with a Wiley Mill (model 3; Arthur H. Thomas Co. Philadelphia, PA), and then further ground to 1 mm particle size using an ultracentrifuge mill (Brinkman Instruments Co., Westbury, NY). In order to correct analysis to 100% DM, 1 g aliquots of feed samples were dried for 4 h in a 105°C oven. Ash content was determined by incinerating 1 g sample for 8 hours at 450°C in a muffle furnace (AOAC 17th ed., method 942.05). Organic matter (OM) was calculated as OM = (100 - % Ash). Samples were analyzed for nitrogen content via Dumas combustion analysis (AOAC 2002, method 968.06), on a Rapid N Cube (Elementar Analysensysteme, GmbH, Hanau Germany). Nitrogen content was then multiplied by 6.25 to calculate CP. Neutral detergent fiber (NDF; Van Soest et al., 1991) and acid detergent fiber (ADF; Robertson and Van Soest, 1981) were analyzed sequentially using the Ankom 200 fiber analysis system (Ankom Technology Corp., Fairport, NY). For NDF, heat-stable alpha-amylase and sodium sulfite were used. Diethyl ether and petroleum ether were used in separate analyses to determine ether extract (EE; AOAC 2002, method 920.39) in an Ankom XT10 fat analysis system (Ankom Technology Corp., Fairport, NY). Non-fibrous carbohydrate was calculated as % NFC = 100 – (% Ash + % CP + % NDF + % EE) according to the NRC (2001).

Dried and ground samples of grass hay and RFDDGS were further composited into four or five month composites and sent to a commercial laboratory (Dairyland Laboratories, Inc. Arcadia, WI) for analysis of minerals and starch. Mineral composition analyses included Ca, P, K, Mg, S, Zn, Mn, Fe, Na, Cl, and Mo. Mineral content, excluding chloride, was determined using inductively coupled plasma spectroscopy (AOAC International, 1995). Chloride content was determined using a direct reading chloride analyzer (Corning 926, Corning Inc., Corning, NY). Starch was analyzed using a modified procedure analyzing glucose using YSI Biochemistry Analyzer (YSI Inc., Yellow Springs, OH; Bach Knudsen, 1997).

Metabolites (cholesterol, glucose, PUN, and triglycerides) were analyzed with commercially available enzymatic or colorimetric assay kids on a micro-plate spectrophotometer (Cary 50, Varian Inc., Walnut Creek, CA). Total plasma cholesterol was

analyzed using cholesterol esterase and oxidase (Cat. #C7510; Pointe Scientific, Inc., Canton, MI) as described by Allain et al. (1974). Serum glucose was analyzed using glucose oxidase as described by Trinder (1969) (Cat. #G7521; Pointe Scientific, Inc., Canton, MI). Plasma urea nitrogen was analyzed using diacetylmonoxime (Procedure 0508; Stanbio Laboratory, Boerne, TX). Plasma triglyceride concentration was analyzed using glycerol phosphate oxidase after hydrolysis by lipoprotein lipase as described by Fossati and Prencipe (1982) that paired the reaction with the classic Trinder (1969) reaction.

For metabolic hormones (IGF-1, insulin, and leptin) samples were analyzed by radio immunoassay (RIA). Samples were sent to the University of Missouri for IGF-1 and leptin analysis. Leptin was analyzed by a highly sensitive ovine leptin RIA, which was validated for bovine plasma (Delavaud et al., 2000). All samples were analyzed within one assay.

During week 16, an extra blood sample was harvested from each heifer as previously described for plasma fatty acid determination. Plasma lipid extractions were performed as described by Bligh and Dyer (1959). Extracted lipids were then prepared for fatty acid analysis using butylation methods as described by Sukhija and Palmquist (1988) with adaptations by Abdelqader et al. (2009). Feed samples for fatty acid analysis were collected and four or five month composites of RFDDGS and grass hay were analyzed for fatty acid profiles via direct butylation techniques (Abdelqader et al., 2009). All prepared fatty acid samples were analyzed via GC (Hewlett Packard 6890, Palo Alto, CA) as described by Abdelqader et al. (2009).

To determine onset of puberty additional blood samples were taken for progesterone analysis. Sampling began when heifers reached 200 kg of BW and continued until presence of a corpus luteum (CL) was confirmed via ultrasonography. Blood samples were taken via coccygeal venipuncture into vacutainer tubes containing K_2EDTA twice weekly (Tuesday and Friday) approximately 4 h post-feeding. Plasma was harvested as previously described. After the presence of a CL was confirmed via ultrasonography indicating that ovulation had occurred, blood samples were no longer taken. Plasma progesterone concentrations were determined using a validated RIA procedure as described by Engel et al. (2008). Pre-cycling baseline progesterone concentrations were 0.55, 0.52, and 0.67 ng/mL for 30DG, 40DG, and 50DG, respectively, SEM = 0.089, P = 0.13). Heifers were determined to have reached puberty when progesterone concentrations were greater than 1 ng/mL, indicating that a CL had formed and the estrous cycle and ovulation had begun.

Rumen fluid samples preserved with sulfuric acid were thawed and centrifuged at 30,000 × g for 20 minutes (Centrifuge: Eppendorf 5403, Eppendorf North America, Hauppauge, NY) and analyzed for ammonia N using a colorimetric assay read on a micro-plate spectrophotometer (Cary 50, Varian Inc.,Walnut Creek, CA.) according to Chaney and Marbach (1962). Rumen fluid samples that were preserved with metaphosphoric acid were thawed and centrifuged at 30,000× g for 20 minutes and analyzed for acetate, propionate, butyrate, isobutyrate isovalerate, and valerate concentrations of VFA were measured using an automated

GC (model 6890; Hewlett-Packard Co., Palo Alto, CA) using a flame-ionization detector. Volatile fatty acids were separated on a capillary column (15 m × 0.25 mm i.d.; Nukol, 17926-01C; Supelco Inc., Bellefonte, PA) using 2-ethylbutyrate as an internal standard. The split ratio of 30:1 in the injector port was at a temperature of 250°C with flow rate of 1.3 mL/min of helium. The column and detector temperature were maintained at 140°C and 250°C, respectively.

Fecal and orts samples for each heifer were composited on an as-is basis by volume. Aliquots of 100 mL of fecal samples were taken from each time point and composited. There were very few orts samples because the heifers were limit-fed and most of the orts samples were only from a single day during the collection period. Orts were composited based on proportions of weight from each day for the few heifers that had orts on multiple days. Samples were then dried and ground as previously described for feed samples. Fecal samples were analyzed for DM, ash, CP, NDF, and ADF as previously described for feed samples. Acid detergent insoluble ash analysis was conducted on all feed composites, fecal samples, and any orts. The method for ADIA analysis consists of analyzing the sample for ADF content (Robertson and Van Soest, 1981) and then determining the ash content using a modified procedure of the AOAC 17th ed., method 935.29. Digestibility calculations were determined according to Merchen (1988).

Statistical Analysis

All data was analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Data was compiled for the monthly feed composite analysis and fatty acid composition, and standard errors were calculated using the MEANS procedure in SAS. Total dietary nutrient values were calculated based on analysis of grass hay and RFDDGS for each treatment over the course of the study.

Heifer intake, growth, and metabolic profile data were analyzed as a randomized complete block design with repeated measures using the MIXED procedures of SAS (Littell, 2006). The model included treatment, week, and treatment × week interactions. Initial body size measurements, BW, and metabolites were included as covariates within the model. Repeated measures by week of the feeding period were done on intakes, BW, body measures, and plasma metabolites using block as the subject. Akaike's criterion was used to determine the most suitable covariance structure in repeated measures for each parameter. Significant differences among treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$. Linear and quadratic effects of treatments were analyzed using orthogonal contrasts.

Regression procedures of SAS were used to determined average change per day for ADG and body frame growth. The *P values* for the interaction term of treatment and time using MIXED analysis were used to determine significance among treatments (Kutner et al., 2004).

Gain to feed ratio was calculated as the ratio of average daily gain (slope of BW regression) to DMI for each treatment.

The MIXED procedures of SAS were used for the analysis of plasma fatty acid profile and concentration and total tract digestibility of nutrients. The model included only treatment with block included as a random variable. Fecal concentrations of nutrients and the internal marker were used to calculate estimates of fecal outputs.

Puberty data was analyzed as binomial data (cycling or not cycling) by certain criteria for age or weight. Puberty was also analyzed using repeated measures by 10 d and 10 kg intervals of age and BW.

D. RESULTS AND DISCUSSION (NOTE: All tables and figures can be found at the end of this report.)

Feed Analysis

The nutrient composition of the individual ingredients used in the experimental diets is presented in Table 2. Because the RFDDGS was purchased in two large batches, nutrient composition of the RFDDGS did not vary much over the duration of the study. However, there was some variation in the nutrient composition of the grass hay over the duration of the experiment. Variation in the hay was because changes in weather and humidity.

Average nutrient composition of the experimental diets is presented in Table 3. The nutrient composition was based on individual ingredient analysis over the course of the study. Overall, the nutrient composition of the treatment diets was on target with the objective of this research. The dietary CP concentration was formulated to increase with increasing concentrations of RFDDGS because the CP was expected to be used as energy by the heifers. Formulated CP values were 16.5, 19.4, and 21.3% for 30DG, 40DG, and 50DG, respectively and actual experimental diets were very close to these values. Ether extract concentration of the diets also increased with increasing concentrations of RFDDGS. Diets were formulated to be 3.5, 4.0, and 4.5% EE for 30DG, 40DG, and 50DG, respectively. Actual experimental diets were slightly less than formulated due to lesser EE in the RFDDGS than originally expected. Neutral detergent fiber decreased with increasing concentrations of RFDDGS as expected by experimental design. Experimental diets were formulated to be 52.8, 48.3, and 45.2% NDF for the 30DG, 40DG, and 50DG diets, respectively. Experimental diets had greater NDF than formulated due to changes in grass hay quality over the duration of the study. Starch concentration increased with increasing dietary concentration of RFDDGS; however, starch concentrations were very low across all diets. Therefore, as expected by experimental design, diets had increased concentrations of CP and EE, but low starch concentrations meaning that fat, fiber, and protein rather than starch were the energy sources in the diets. In the current study, limit-feeding was used as the feeding strategy to avoid overconsumption of nutrient and

energy that would result in overweight heifers. As mentioned, heifers were limit-fed at 2.65, 2.50, and 2.35% of BW for the 30DG, 40DG, and 50DG treatments, respectively. As the concentration of RFDDGS in the diet increased, the nutrient and energy density of rations increased so amount fed as percentage of BW was decreased to target similar total intakes of CP and energy. Because the consumption of bedding material can be a concern when limit-feeding, pens were only bedded once every 2 wk on the day after body measurements and samples were collected to avoid consumption of bedding material and interference with data collection.

Differences in nutrient composition of the diets were further reflected in nutrient intake (Table 4). Crude protein, EE, starch, and sulfur intake increased with increasing concentration of RFDDGS; however, NDF intake decreased. Because starch concentrations were so low this shows that protein and fat were used for energy. Sulfur intake increased across treatments; however, sulfur toxicity was never a concern. Sodium bicarbonate and limestone which contains calcium carbonate were also included in the experimental diets to buffer the rumen and help minimize the risk of sulfur toxicity. Additionally, water supplied to heifers was from a municipal water treatment plant and had low sulfate concentration (approximately140 mg/kg) compared to local well water which varies in sulfate concentration by location. According to calculations using NRC (2001) software when nutrient compositions of feedstuffs were entered after analysis, metabolizable energy (ME) intake was similar among treatments with a small numerical decrease as the proportion of RFDDGS in the diet increased. However, net energy gain ($NE_{g)}$ intake numerically increased as the proportion of RFDDGS increased in the diet. This could be because of less energy required to breakdown the forage portion of the diet for use by the heifers.

Table 5 shows the fatty acid profiles of the RFDDGS and grass hay used in the experimental diets. Grass hay had greater concentrations of medium and long chain fatty acids (C10:0, C12:0, C12:1, C16:1, C20:0, and C18:3 α), while RFDDGS had greater concentrations of total and long chain fatty acids (C14:0, C16:0, C18:1 *cis* 11, and C18:2 *cis* 9, *cis* 12). Fatty acid profiles of the experimental diets are found in Table 6. There were more total and long chain fatty acids (C16:0, C18:1 *cis* 11, and C18:2 *cis* 9, *cis* 12) as dietary concentrations of RFDDGS increased. Differences in fatty acid profiles of the diets were further reflected in the fatty acid intake (Table 6). Intakes of medium chain fatty acids (C10:0, C12:0, C12:1) linearly decreased with increasing concentrations of RFDDGS. However, intake of long chain (C14:0, C16:0, C18:1 cis 11, and C18:2 cis 9, cis 12) increased with increasing concentrations of RFDDGS. This is of interest because linoleic acid (C18:2) is a precursor for arachidonic acid (C20:4) which is used in the synthesis of prostaglandins (Funston, 2004) and may play a role in the onset of puberty.

Heifer Growth Performance

There were no treatment by week interactions for any of the heifer performance parameters measured. Body weight, DMI, and gain: feed are presented in Table 8. Heifer BW and ADG were similar among treatments and increased over the course of the experiment. This was anticipated because heifers were limit-fed based on a percentage of BW. However, ADG in this experiment was greater than the 0.8 kg/d than is recommended (Zanton and Heinrichs, 2005). There was a slight numerical increase in ADG as the percentage of RFDDGS increased. This supports that finding that ME intake was similar across treatment, but the NE_g increased as RFDDGS was included at a greater proportion of the diet. The NRC (2001) software was used to formulate the diets. The results from this experiment and Anderson et al. (2015a) suggest that the software overestimates the energy requirements of growing dairy heifers or underestimates energy provided by DDGS. The current experiment and Anderson et al. (2015a) diets containing DDGS show that heifers can be limit-fed to control ADG, but the feeding rate must be less than what the NRC (2001) recommends to achieve the 0.8 kg/d ADG recommended by Zanton and Heinrichs (2005). Similar ADG among treatments indicate that heifers were all at similar planes of nutrition despite different nutrient compositions, intakes, and dietary energy sources.

Dry matter intake decreased and gain: feed increased across treatments, because nutrient density of the diet also increased with increased RFDDGS, resulting in less feed needed to be offered to obtain target ADG. This difference in DMI is consistent with what is reported in other experiments that controlled the nutrient intake in diets differing in forage concentration (Hoffman et al., 2007; Lascano and Heinrichs, 2009; Zanton and Heinrichs, 2009). Diets with greater forage concentration are not as nutrient dense and more of the DM must be consumed in order to maintain nutrient intake. As originally hypothesized, 50DG had the greatest gain: feed. Since a limit-feeding strategy was utilized in this experiment, heifers fed 50DG were able to maintain ADG while consuming less feed. The ME and NEg intake results would also suggest that heifers fed 50DG were more efficient in energy utilization and able to directing more energy to gain than to other requirements.

Frame size measurements and BCS are presented in Table 9. Heifers had similar predicted transmitting ability for type composite score (1.25, 1.09, and 1.20 for 30DG, 40DG, and 50DG, respectively, SEM = 0.107, $P = 0.57$) based on genomic testing; therefore it was not used as a covariate term for growth performance. There were no treatment by week effects for any of the parameters measured. All frame growth parameters increased over the course of the experiment and had significant week effects. There were also no differences in change per day for any of the frame growth measurements, suggesting that treatments diets provided enough CP and energy to maintain consistent growth over the experiment. A treatment effect was observed for withers height and paunch girth with 40DG having the greatest average measurements. Although withers height and paunch girth were greatest for the 40DG treatment, differences were numerical and biologically very small.

There was also a treatment effect for BCS (Table 9) with 40DG having the greatest score and least 50DG; however, once again differences were numerically very small. Throughout the experiment, heifers maintained a BCS close to 3.0, indicating that heifers were not accumulating excess adipose tissue. Heifers in this experiment also had large BW and frame sizes for their ages (Heinrichs and Losinger, 1998). Anderson et al. (2015a) also found that heifers limit-fed DDGS at up to 30% of dietary DM had small differences in BCS and maintained frame growth even with greater ADG (0.96 kg/d) than the recommended 0.8 kg/d by Zanton and Heinrichs (2005).

Rumen Fermentation

Rumen fermentation characteristics are presented in Table 10. There was a treatment by week interaction for isobutyrate concentration and a tendency for a treatment by week interaction for acetate, valerate, and total VFA concentrations. Propionate concentration linearly increased as the dietary concentration of RFDDGS increased, while butyrate concentration, acetate to propionate ratio, and pH linearly decreased with increasing dietary concentration of RFDDGS. As the concentration of RFDDGS increased the propionate molar percentage also increased, while acetate and butyrate molar percentages decreased. The shift in molar VFA concentrations in the present study is a result of differences in forage concentration in the experimental diets, suggesting a shift in bacterial species population in the rumen. Acetate production within the rumen is due to the fermentation of structural carbohydrates by cellulolytic bacteria, while propionate formation is due to the fermentation of nonstructural carbohydrates by amylolitic bacteria (Enjalbert et al., 1999). The decrease in acetate to propionate ratio as concentration of RFDDGS increased is consistent with other studies that fed heifers diets differing in concentrate proportions (Lascano et al., 2009; Suarez-Mena et al., 2015). It is also indicates that fermentation may have been more energy efficient in the heifers fed greater proportions RFDDGS and coincides with the finding that a larger portion of ME intake could be directed towards growth.

Rumen ammonia-N concentration linearly increased as the dietary concentration of RFDDGS increased. Suarez-Mena et al. (2015) fed increasing concentrations of DDGS in replacement of canola meal at two forage concentrations and found that NH_3-N tended to be greater for high forage diets because of lower microbial activity. However, diets in that experiment had greater starch and NFC concentrations and less CP than diets in the current experiment. Ammonia is used for protein synthesis within the rumen and it accumulates when protein degradation exceeds the ability of microbes to assimilate amino acids and NH_3 (NRC, 2001). The supply of fermentable carbohydrates affects the assimilation of N by rumen bacteria (Nocek and Russell, 1988; Bach et al., 2005) and microbial growth is determined by synchrony between N and carbohydrates (Bach et al., 2005). The low carbohydrate concentrations in the experimental diets and the increased CP concentrations may explain the

greater NH_3-N across treatments. Overall, increasing concentrations of RFDDGS, did not appear to negatively affect fermentation enough to have changes growth performance and may have shifted fermentation towards more efficient energy utilization in in the current study.

Total Tract Nutrient Digestion

Total tract nutrient digestibility that was evaluated in week 16 of the study is presented in Table 11. Digestibility of NDF and ADF was similar among treatments, whereas digestibility of DM, OM, and CP linearly increased with increasing concentrations of RFDDGS with the 50DG having the greatest digestibility of these nutrients ($P < 0.01$). Increases in DM and OM digestibility are consistent with what was reported by Suarez-Mena et al. (2015) when dairy heifers were fed increasing concentrations of DDGS in replacement of canola meal with two different forage concentrations. However, that study found a quadratic effect on DM and OM digestibility with the greatest digestibility occurring when DDGS was included at 14% of dietary DM, regardless of forage concentration. It was suggested that at 14% of the diet, the fat content in the diet was low enough and rumen microbes were still able to saturate FA. At greater dietary concentrations of DDGS, the greater amounts of fat consumed may have interfered with fermentation because of the effects of unsaturated lipids on microbial growth and negatively affected the digestibility of non-lipid energy sources (Jenkins, 1993; NRC, 2001). Since RFDDGS was utilized and diets were limit-fed in the current experiment, the fat content in the rumen never reached great enough concentrations to have negative effects on the digestion of other nutrients.

Anderson et al. (2015) fed growing dairy heifers low or high fat DDGS at approximately 22 or 34% of dietary DM and found differences in CP, NDF, and ADF digestibility. It was speculated that because of the low starch concentration in the traditional full fat DDGS diet there may have been better fiber utilization within the rumen (Anderson et al., 2015a). Ranathunga et al. (2012) also found that NDF digestion improved in mature dairy cows fed high forage diets containing DDGS when compared to low forage diets containing DDGS. They speculated that the fat from DDGS was slowly introduced into the rumen and less severe effects on rumen fermentation because the DDGS was bound in the feed particle. However, in contrast to the finding of the previous research there were no differences in fiber digestibility in the current study.

The amount of CP digestion in the current study is consistent with previous research (Kleinschmit et al., 2007; Anderson et al., 2015a). Kleinschmit et al., (2007) conducted an in situ experiment followed by in vitro intestinal digestion procedures and found that total digestible protein in DDGS ranged from 70.7% to 84.9%. In the current experiment, the total tract digestibility of CP was 86%, which is slightly greater than that reported by Kleinschmit et al. (2007), the 30DG and 40DG treatments were however within the range. Because the diets contained low starch concentrations, the utilization of CP was expected to be improved.

Fecal Nutrient Output

Nutrient intakes and calculated estimates for fecal output for the heifers during the 3 day collection period for total tract digestibility of nutrients are shown in Table 12. Nutrient intakes of CP and N linearly increased, whereas intakes of DM, OM, and NDF linearly decreased, with increasing dietary concentration of RFDDGS. Despite differences in nutrient intakes, there were no differences in estimated total fecal nutrient output based on fecal sample composition. The NRC (2001) estimates the dietary requirement for a 250 kg heifer with an ADG of 0.8 kg/d is 129.95 g/d of N. The estimated N intake in the current study linearly increased across treatments and was almost double what the NRC (2001) estimates as the dietary requirement, however, there were no changes in fecal N output among treatments. It is speculated that because of the low starch concentration of the diets and lack of gluconeogenic precursors from starch that the extra CP and AA in the diets may be partially used for gluconeogenesis. Lascano et al. (2009) conducted a study in which dairy heifers were fed either high or low concentrate diet with or without yeast culture supplementation. In this experiment diets were approximately 13% CP and N intake average approximately 134 g/d among concentrate levels with fecal N being approximately 60 g/d among concentrate levels. The increased CP % in the diets of the current study explains the linear increase in total N intake among treatments. Results demonstrate that despite increase in N intake, with limit-feeding strategies, fecal N output will be only minimally impacted by up to 50% inclusion rate of RFDDGS compared to 30% inclusion rate. Although more research is necessary to determine effects on urinary N outputs.

Metabolic Profile and Puberty

Average plasma fatty acids proportions (mg/100 mg of FA) and concentrations (µg/ mL of plasma) are presented in Tables 13 and 14, respectively. There was a quadratic effect ($P = 0.04$) and linear tendency ($P = 0.07$) to increase the proportion of total fatty acids and linoleic acid (C18:2) with increased dietary inclusion of RFDDGS. Linoleic was also the greatest proportion of fatty acids across all treatments. Plasma concentration of linoleic acid also linearly increased ($P = 0.01$) as more RFDDGS was included in the diet. All heifers also had a large proportion of plasma fatty acid as oleic acid (C18:1 *cis* 9), but plasma concentrations were not affected by treatment. Plasma concentration of palmitic acid (C16:0) linearly increased with increasing concentrations of RFDDGS as expected by experimental diets. There was also a linear ($P = 0.03$) and quadratic ($P = 0.04$) effect for arachidonic acid (C20:4) which is the precursor for synthesis of prostaglandins (Funston, 2004) and may play a role in the onset of puberty. Overall results for fatty acids analysis demonstrated that total fatty acid and polyunsaturated fatty acid (PUFA) concentration in the blood was linearly increased ($P < 0.01$) with a quadratic effect ($P = 0.01$) as dietary inclusion rate of RFDDGS increased. Meaning there was a marked increase of

plasma total fatty acids and PUFA in the heifers fed 50DG, and less of a difference between heifers fed the 30DG or 40DG diets. The metabolic effects of these fatty acid changes in dairy heifers are not yet fully understood.

Blood metabolite and metabolic hormone concentrations are presented in Table 15. There were no treatment by week interactions for any of the metabolites or metabolic hormones measured. Despite differences in total plasma fatty acid concentrations, there were no differences in concentrations of plasma triglycerides (Table 15) which are comprised of fatty acids chains and a glycerol backbone and a major storage form of fat in the body. However, there was a quadratic effect for plasma cholesterol (Table 15). Cholesterol is an important metabolite in reproduction because it is a precursor for steroid hormone synthesis. Progesterone, a steroid hormone, may be easily affected by plasma cholesterol concentration. However, due to the scope of this study, progesterone was not sampled at the frequency necessary to monitor concentrations throughout the estrous cycle. Concentrations of serum glucose (Table 15) did not differ across treatments. There was a treatment effect for PUN with concentrations linearly increasing with increasing concentrations of RFDDGS. This can be explained by the increase in dietary crude protein concentrations across treatments.

There were no differences in concentrations of plasma insulin or leptin (Table 15); however, there was a quadratic effect for IGF-1 concentration (Table 15). The greatest concentrations of IGF-1 were found in the 30DG and 50DG treatments, IGF-1 is capable of activating insulin receptors at great concentrations; however, no differences were reflected in plasma insulin concentrations. Anderson et al. (2015b) limit-fed DDGS at up to 30% of dietary DM and found no differences in plasma insulin suggesting that short-term energy status was maintained by feeding DDGS. Long-term energy was maintained as demonstrated by plasma leptin concentrations across treatments (Zieba et al., 2005). This is in agreement with previous research that altered dietary fat concentrations in beef (Garcia et al., 2003) and dairy heifers (Block et al., 2003; Anderson et al., 2015b). The maintenance of short and long-term energy status suggests that heifers are using the fat and protein from RFDDGS as energy to maintain growth in replacement of forage fiber and protein when utilizing a limit-feeding strategy.

Age and BW at puberty are shown in Table 16. Despite no differences in age or BW at puberty, values follow a similar numerical pattern as plasma cholesterol, a precursor to reproductive hormones, with 40DG having the least plasma cholesterol concentrations as well as numerically the greatest age and BW at puberty. Percentage of heifers cycling over time by age and BW are presented in Figures 1 and 2. There was a treatment by age interaction on onset of puberty. Attainment of puberty is thought to be correlated to body fat content (Zieba et al., 2004; Perry, 2011), but circulating plasma cholesterol and fatty acids may also play a role. Because of similar plasma leptin concentrations, circulating plasma cholesterol and fatty acids may have played a larger role, but more research is necessary to confirm this speculation.

E. BENEFITS TO MINNESOTA ECONOMIC DEVELOPMENT

Increasing the utilization of DDGS is mutually beneficial to the corn ethanol industry and the dairy industry. It benefits corn growers by increasing the market for the corn ethanol co-products. Minnesota is ranked as the 7th greatest dairy production state with approximately 460,000 dairy cows and neighboring eastern South Dakota, Northern Iowa, and Wisconsin all having strong or growing dairy sectors. Therefore, increasing the use of DDGS by the dairy industry could have large economic impacts for the corn ethanol industry in Minnesota. It also benefits dairy producers by providing opportunity to increase the utilization of DDGS which cost less compared to soybean meal and other commonly used protein sources, thus decreasing the overall rearing costs for replacement dairy heifers.

In the current project it was estimated using dry matter intake results that the average cost of feeding the 30DG was $0.88, 40DG was $0.87 and, 50DG was $0.79 per heifer per day during the age range on the study (7 to 11 months). This cost decrease was because as heifers were offered one additional pound of RFDDGS in the diet approximately 2 pounds of grass hay could be removed from the ration. Therefore, as long as the cost of 1 ton of RFDDGS does not exceed the cost of 2 tons of hay, producers should have maintained or decreased costs by feeding increasing the amount of RFDDGS in replacement of forage fed to growing dairy heifers in limit-fed or target-fed rations. Overall, this research demonstrated a method to utilize more RFDDGS to decrease heifer feeding costs while maintaining growth performance and increasing feed efficiency.

F. MARKETING (EDUCATION, OUTREACH, AND PUBLICATIONS)

Manuscripts in Peer-Reviewed Journals:

1. Manthey, A. K., J. L. Anderson, and G. A. Perry. 2016. Feeding reduced-fat distillers dried grains in replacement of forage in limit-fed dairy heifer rations: Effects on growth performance, rumen fermentation, and total tract digestibility of nutrients. Journal of Dairy Science. *Submitted – in review*.

2. Manthey, A. K., J. L. Anderson, G. A. Perry† and D.H. Keisler. 2016. Feeding reduced-fat distillers dried grains in replacement of forage in limit-fed dairy heifer rations: Effects on metabolic profile and puberty. Journal of Dairy Science. *In final preparation.*

Workshop Presentations:

1. Jill Anderson. Heifers Diets and DDGS feeding consideration. I-29 Dairy Consortium Workshop. January 5-8, 2015 in Orange City IA, Brookings SD, Fergus Fall MN, and Mandan ND. (Growth performance results were shared as part of this talk.)

SDSU Extension e-articles:

1. Angela Manthey and Jill Anderson. 12/1/2015. Reduced-Fat Distillers Grains: How much can we feed to growing dairy heifers? iGrow.org – Livestock – Dairy – Innovation/Research. http://igrow.org/livestock/dairy/reduced-fat-distillers-grains-how-much-can-we-feed-to-growing-dairy-heifers/#sthash.79hym4Pc.dpuf

2. Angela Manthey and Jill Anderson. TBD. Reduced-Fat Distillers Grains: Effect on rumen fermentation and total tract digestibility in growing dairy heifers. iGrow.org – Livestock – Dairy – Innovation/Research. *In final preparation*.

3. Angela Manthey, Jill Anderson, and George Perry. TBD. Reduced-Fat Distillers Grains: Effect on metabolic profile and on set of puberty in growing dairy heifers. iGrow.org – Livestock – Dairy – Innovation/Research. *In final preparation*.

Radio interviews/spots:

1. Jill Anderson. MCGA Radio. Aired 12/2014 on Linder Farm Network and Red River Farm Network. https://www.youtube.com/watch?v=elBUWida6qg&feature=youtu.be

2. Angela Manthey. igrow (SDSU Extension) Radio. Aired 12/3/2015 on Dakota Farm Talk. http://podcast.sdstate.edu/groups/igrowradio/weblog/c6a65/Feeding_reduced_fat_DDGS_to_growing_dairy_heifers.html

Scientific Research Conference Presentations:

1. Manthey, A. K., J. L. Anderson, G. A. Perry. 2015. Growth Performance of dairy heifers fed reduced-fat distillers grains in replacement of forage in limit-fed rations. J. Dairy Sci. 98: Suppl. 2: 459 (Abstr. T415). Joint Annual Meeting of American Society of Animal Science and the American Dairy Science Association in Orlando, FL, July 2015.

2. Manthey, A. K., J. L. Anderson, G. A. Perry, and D. H. Keisler. 2015. Metabolic profile and onset of puberty in dairy heifers fed reduced-fat distillers grains in replacement of forage. J. Dairy Sci. 98: Suppl. 2: 735 (Abstr. W329). Joint Annual Meeting of American Society of Animal Science and the American Dairy Science Association in Orlando, FL, July 2015.

Other Research Meetings:

1. Oct 8-11, 2014: Lincoln, NE. Jill Anderson attended the "North Central Cooperative Research Project NC-2042. Management Systems to Improve Economic and Environmental Sustainability of Dairy Enterprises" group meeting. This a multi-state project of dairy nutrition faculty from a large number of universities around the country. Presented results on heifer growth performance as part of the South Dakota Agricultural Experiment Station Report.

2. Oct 19-23, 2015: Barcelona, Spain. Jill Anderson attended the "North Central Cooperative Research Project NC-2042. Management Systems to Improve Economic and Environmental Sustainability of Dairy Enterprises" group meeting. This a multi-state project of dairy nutrition faculty from a large number of universities around the country. At this meeting the NC 2042 group met with a cross-section of professionals and researchers involved with the European and Spanish Dairy Industry. Presented results on heifer metabolic profile and on set of puberty as part of the South Dakota Agricultural Experiment Station Report.

G. CONCLUSIONS

As originally hypothesized, heifers were able to be limit-fed diets containing RFDDGS at up to 50% of dietary DM and maintain growth performance. There were no differences in BW and ADG was maintained across treatments. However, ADG was greater than recommended for all treatments, but heifers did not accumulate excess adipose tissue as demonstrated by small increases overtime in BCS and leptin. In addition, increasing the dietary concentration of RFDDGS in replacement of forage increased gain: feed and nutrient digestibility of DM, OM, and CP. This research demonstrates that RFDDGS can be utilized in limit-fed rations for growing dairy heifer at up to 50% of the diet, which is a much greater dietary concentrations than previously researched or recommended. High concentrations of rumen ammonia and plasma urea nitrogen do raise some caution against feeding above 50% of dairy heifer rations as RFDDGS. Rough cost estimates demonstrated that feeding increased amounts of RFDDGS can maintain or decrease the overall cost of the heifers depending on the price of forages. **Overall, this research demonstrated a method to utilize more RFDDGS to decrease heifer feeding costs while maintaining growth performance and increasing feed efficiency.** Results of this research have been communicated to the public, dairy industry, and scientific community through variety of ways. Communication (publication) of results is still in progress, but will be completed early in 2016.

H. FUTURE NEEDS AND PLANS

Research specifically on feeding DDGS to growing dairy heifers is limited. Also, the majority of research on feeding DDGS to dairy cattle has focused on full-fat DDGS. Advancements in biofuel production have created a new generation of reduced-fat DDGS (RFDDGS) and low-fat DDGS for which new feeding guidelines need to be developed for dairy replacement heifers. This project with MCR&PC and AURI found that up to 50% of heifer diets can be fed as RFDDGS, replacing all other concentrate ingredients and some forage, when using limit-feeding strategies for the total ration. A proposal of research that builds on these findings was recently submitted to the MCR&PC. It has been observed that some dairy producers prefer to feed heifers a grain mix and allow free-choice hay consumption with bale feeders. In this recently proposed project, RFDDGS will be limit-fed or target-fed at increasing rates while forage will be provided ad libitum. It is hypothesized that feeding increased amounts of RFDDGS will stimulate satiety signals that will cause heifers to decrease forage intake on their own, improving feeding efficiency and nutrient utilization. If funded, this hypothesis will be tested by conducting a 16-week feeding trial with forty-eight heifers fed one of three feeding rates of RFDDGS based % of body weight and determining effects on forage intakes, growth performance, gain to feed and other parameters related to growth and development.

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Table 1. Ingredient composition of treatment diets with increasing inclusion amounts of reduced-fat distillers dried grains with solubles (**RFDDGS**) in replacement of forage limit-fed to growing replacement Holstein heifers.

	Treatment[1]		
Item[2]	30DG	40DG	50DG
Ingredient, % DM			
RFDDGS	30.0	40.0	50.0
Grass hay	68.5	58.5	48.5
Vitamin and mineral premix[3]	0.75	0.75	0.75
Limestone	0.30	0.30	0.30
Sodium bicarbonate	0.30	0.30	0.30
Salt	0.15	0.15	0.15

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Formulated using NRC, 2001.

[3]Contained: 2.2 g/kg of lasalocid, 14.5% Ca, 8.0% P, 21.0% NaCl, 2.5% Mg, 1.5% K, 2.0% S, 4,100 mg/kg Mn, 1,250 mg/kg Cu, 70 mg/kg Co, 70 mg/kg I, 53 mg/kg Se, 5,500 mg/kg Zn, 325 mg/kg Fe, 704,000 IU/kg Vitamin A, 140,800 IU/kg Vitamin D_3, and 5,280 IU/kg Vitamin E (Future Cow Supreme Premix B2000, Land O' Lakes, Inc., St. Paul, MN).

Table 2. Nutrient composition of the grass hay and RFDDGS used in the treatment diets fed to growing Holstein heifers.

	Grass hay		RFDDGS	
Item[1]	Mean	SE	Mean	SE
DM[2], %	86.3	0.314	86.9	0.347
Ash[2]	8.76	0.328	4.68	0.037
OM[2]	91.2	0.328	95.3	0.034
CP[2]	9.81	0.417	33.6	0.175
ADF[2]	37.8	0.495	10.0	0.350
NDF[2]	66.4	0.619	29.8	0.381
EE (Diethyl)[2]	1.87	0.101	12.9	0.131
EE (Petroleum)[2]	1.05	0.102	7.80	0.079
NFC[2,3]	14.0	0.903	24.1	0.331
Starch[4]	0.84	0.033	6.00	0.041
Ca[4]	0.37	0.053	0.07	0.003
P[4]	0.20	0.028	0.86	0.017
S[4]	0.15	0.009	0.73	0.007

[1] % DM, unless otherwise indicated.
[2] Results from analysis of monthly composites (n=13).
[3] %NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001).
[4] Results from analysis of four- or five-month composites (n=4).

Table 3. Nutrient composition treatment diets with increasing inclusion amounts of RFDDGS in replacement of grass hay limit-fed to growing Holstein heifers.

	Treatment[1]					
	30DG		40DG		50DG	
Item[2], % DM	Mean	SE	Mean	SE	Mean	SE
DM[3], %	86.7	0.29	86.7	0.29	86.8	0.29
OM[3]	91.1	0.23	91.5	0.19	91.9	0.16
Ash[3]	8.83	0.226	8.42	0.194	8.02	0.162
CP[3]	16.8	0.32	19.2	0.29	21.5	0.26
ADF[3]	28.9	0.41	26.1	0.39	23.3	0.37
NDF[3]	54.4	0.47	50.8	0.43	47.1	0.40
EE (Diethyl)[3]	5.17	0.077	6.27	0.076	7.38	0.078
EE (Petroleum)[3]	3.06	0.073	3.74	0.066	4.41	0.062
NFC[3,4]	16.8	0.63	17.8	0.55	18.9	0.47
Forage NDF[3]	45.5	0.42	38.8	0.36	32.2	0.30
Non-forage NDF[3]	8.95	0.114	11.9	0.15	14.9	0.19
Starch[5]	2.38	0.022	2.89	0.020	3.41	0.021
Ca[5]	0.28	0.036	0.25	0.031	0.22	0.025
P[5]	0.40	0.015	0.47	0.010	0.54	0.006
Mg[5]	0.21	0.005	0.23	0.004	0.25	0.003
K[5]	1.70	0.191	1.61	0.159	1.52	0.127
S[5]	0.33	0.004	0.38	0.003	0.44	0.002
Na[5]	0.03	0.005	0.03	0.004	0.03	0.003
Cl[5]	0.48	0.083	0.44	0.072	0.40	0.061
ME[6], Mcal/Kg DM	2.27	-	2.39	-	2.51	-
NE_g[6], Mcal/Kg DM	0.81	-	0.90	-	0.99	

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).
[2]% DM, unless otherwise indicated.
[3]Results from analysis of monthly composites (n=13).
[4]% NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001).
[5]Results from analysis of four- or five-month composites (n=3).
[6]Estimated by inputting mean nutrient analysis of feeds into ration formulation program (NRC, 2001).

Table 4. Mean nutrient intakes for Holstein heifers fed increasing inclusion amounts of RFDDGS in replacement of grass hay.

	Treatment[1]				*P-value*[2]				
Nutrient, kg/d	30DG	40DG	50DG	SEM	Trt	wk	Trt × wk	L	Q
DM[3]	6.49	6.21	5.84	0.117	<0.01	<0.01	1.00	<0.01	0.50
OM[3]	5.91	5.68	5.37	0.107	<0.01	<0.01	1.00	<0.01	0.48
CP[3]	1.09	1.19	1.26	0.022	<0.01	<0.01	0.99	<0.01	0.19
NDF[3]	3.53	3.15	2.75	0.060	<0.01	<0.01	0.96	<0.01	0.76
ForageNDF[3]	2.95	2.41	1.88	0.046	<0.01	<0.01	0.09	<0.01	0.84
NonforageNDF[3]	0.58	0.74	0.87	0.014	<0.01	<0.01	0.11	<0.01	0.05
EE (Diethyl)[3]	0.33	0.39	0.43	0.007	<0.01	<0.01	0.76	<0.01	0.13
EE (Petroleum)[3]	0.20	0.23	0.26	0.004	<0.01	<0.01	0.73	<0.01	0.10
Starch[4]	0.15	0.18	0.20	0.003	<0.01	<0.01	0.75	<0.01	0.17
Sulfur[4]	0.021	0.024	0.026	0.0004	<0.01	<0.01	0.90	<0.01	0.15
ME, Mcal/d	14.7	14.8	14.7	0.28	0.61	<0.01	1.00	0.72	0.36
NE_g, Mcal/d	5.25	5.59	5.78	0.105	<0.01	<0.01	1.00	<0.01	0.24

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Significance of effects for treatment (**Trt**), week (**wk**), treatment × week (**Trt × wk**), and linear (**L**) and quadratic (**Q**) orthogonal contrasts.

[3]Results from analysis of monthly composites (n=13).

[4]Results from analysis of four- or five-month composites (n=3).

Table 5. Fatty acid composition of the grass hay and RFDDGS used in the treatment diets fed to growing Holstein heifers.

	Grass hay		RFDDGS	
Fatty acid[1]	Mean	SE	Mean	SE
	----------g/100g----------			
C10:0	5.227	0.8346	0.819	0.0520
C12:0	3.603	0.3962	0.519	0.0153
C12:1	10.391	0.7452	0.695	0.0123
C14:0	1.271	0.0661	5.048	0.0327
C16:0	7.361	0.3006	12.520	0.0597
C16:1	5.809	0.3458	0.136	0.0029
C18:0	0.780	0.0662	1.793	0.0078
C18:1, *cis* 11	1.351	0.1353	17.341	0.0465
C18:1, *trans* 11	0.096	0.0357	0.739	0.0032
C18:2, *cis* 9, *cis* 12	4.301	0.2969	48.955	0.2342
C18:3 ɣ	0.404	0.0329	0.550	0.2397
C20:0	18.192	1.0332	4.763	0.0423
C18:3 α	22.784	0.8043	3.498	0.0363
C18:2 *trans*[2]	1.463	0.0855	0.165	0.0036
C20:4	0.425	0.1275	0.133	0.0020
Others[3]	16.543	0.3880	2.327	0.0208
	----------g/kg DM----------			
C10:0	1.046	0.1764	0.662	0.0685
C12:0	0.717	0.0757	0.416	0.0063
C12:1	2.069	0.1440	0.559	0.0309
C14:0	0.253	0.0148	4.055	0.1514
C16:0	1.464	0.0286	10.062	0.3909
C16:1	1.160	0.0879	0.110	0.0035
C18:0	0.155	0.0087	1.441	0.0654
C18:1, *cis* 11	0.266	0.0180	13.944	0.6332
C18:1, *trans* 11	0.019	0.0071	0.594	0.0251
C18:2, *cis* 9, *cis* 12	0.856	0.0558	39.341	1.5284
C18:3 ɣ	0.080	0.0043	0.459	0.2034
C20:0	3.629	0.2455	3.831	0.1892
C18:3 α	4.555	0.3130	2.811	0.1118
C18:2 *trans*[2]	0.291	0.0077	0.133	0.0085
C20:4	0.086	0.0272	0.106	0.0047
Others[3]	3.294	0.0326	1.871	0.0789
Total	19.943	0.6649	80.396	3.4871

[1] Represented as number of carbons: number of double bonds.

[2] Includes all C18:2 *trans* isomers.

[3] Sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C11:0, C11:1, C13:0, C13:1, C14:1, C15:0, C15:1, C16:1 *trans*, C17:0, C17:1, C18:1, *trans* 6, C18:1, *trans* 9, C18:1, *trans* 10, C18:1, *cis* 9, C20:1, 5, C20:1, 8, C20:1 *cis*, C18:2, *trans* 10, *cis* 12, C18:2, *cis* 9, *trans* 11, C20:2, 11, 14, C20:3 *homo* ɣ, C22:0, C20:3, 11, 14, 17, C22:1, C23:0, C20:5, C22:2, C24:0, C22:3, C22:4, C24:1, C22:5, N3, C22:6, and unidentified fatty acids.

Table 6. Fatty acid compositions of the treatment diets with increasing inclusion amounts of RFDDGS in replacement of grass hay limit-fed to growing Holstein heifers.

	Treatment[1]					
	30DG		40DG		50DG	
Fatty acid[2], g/kg DM	Mean	SE	Mean	SE	Mean	SE
C10:0	0.915	0.1407	0.877	0.1298	0.839	0.1189
C12:0	0.616	0.0506	0.586	0.0426	0.556	0.0346
C12:1	1.585	0.1074	1.434	0.0960	1.283	0.0846
C14:0	1.390	0.0432	1.770	0.0583	2.151	0.0737
C16:0	4.022	0.1033	4.881	0.1441	5.741	0.1852
C16:1	0.828	0.0612	0.722	0.0527	0.617	0.0442
C18:0	0.538	0.0137	0.667	0.0211	0.796	0.0285
C18:1, *cis* 11	4.366	0.1795	5.734	0.2443	7.102	0.3091
C18:1, *trans* 11	0.191	0.0114	0.249	0.0132	0.306	0.0151
C18:2, *cis* 9, *cis* 12	12.389	0.4375	16.237	0.5930	20.086	0.7488
C18:3 ɣ	0.193	0.0602	0.230	0.0806	0.268	0.1011
C20:0	3.635	0.1575	3.655	0.1368	3.676	0.1233
C18:3 α	3.963	0.2335	3.789	0.2098	3.614	0.1870
C18:2 *trans*[3]	0.239	0.0029	0.223	0.0015	0.207	0.0012
C20:4	0.091	0.0201	0.093	0.0178	0.095	0.0156
Others[4]	2.818	0.0452	2.675	0.0498	2.533	0.0545
Total	37.780	1.4026	43.825	1.6896	49.870	1.9822

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Represented as number of carbons: number of double bonds.

[3] Includes all C18:2 *trans* isomers.

[4] Sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C11:0, C11:1, C13:0, C13:1, C14:1, C15:0, C15:1, C16:1 *trans*, C17:0, C17:1, C18:1, *trans* 6, C18:1, *trans* 9, C18:1, *trans* 10, C18:1, *cis* 9, C20:1, 5, C20:1, 8, C20:1 *cis*, C18:2, *trans* 10, *cis* 12, C18:2, *cis* 9, *trans* 11, C20:2, 11, 14, C20:3 *homo* ɣ, C22:0, C20:3, 11, 14, 17, C22:1, C23:0, C20:5, C22:2, C24:0, C22:3, C22:4, C24:1, C22:5n3, C22:6, and unidentified fatty acids

Table 7. Mean fatty acid intakes for heifers fed increasing inclusion amounts of RFDDGS in replacement of grass hay in limit-fed rations.

	Treatment[1]				*P-value*[2]				
Fatty acid, g/d	30DG	40DG	50DG	SEM	Trt	wk	Trt × wk	L	Q
C10:0	5.94	5.44	4.90	0.103	<0.01	<0.01	1.00	<0.01	0.65
C12:0	4.00	3.64	3.25	0.069	<0.01	<0.01	0.99	<0.01	0.68
C12:1	10.28	8.90	7.50	0.169	<0.01	<0.01	0.74	<0.01	0.89
C14:0	9.02	10.99	12.56	0.206	<0.01	<0.01	0.37	<0.01	0.09
C16:0	26.09	30.29	33.54	0.567	<0.01	<0.01	0.77	<0.01	0.13
C16:1	5.37	4.48	3.61	0.086	<0.01	<0.01	0.27	<0.01	0.94
C18:0	3.49	4.14	4.65	0.077	<0.01	<0.01	0.60	<0.01	0.11
C18:1, *cis* 11	28.32	35.59	41.49	0.667	<0.01	<0.01	0.17	<0.01	0.07
C18:1, *trans* 11	1.24	1.54	1.79	0.029	<0.01	<0.01	0.22	<0.01	0.08
C18:2, *cis* 9, *cis* 12	80.36	100.77	117.33	1.889	<0.01	<0.01	0.18	<0.01	0.07
C18:3 ɤ	1.25	1.43	1.57	0.027	<0.01	<0.01	0.86	<0.01	0.15
C20:0	23.58	22.69	21.47	0.426	<0.01	<0.01	1.00	<0.01	0.48
C18:3 α	25.71	23.51	21.11	0.444	<0.01	<0.01	1.00	<0.01	0.66
C18:2 *trans*[3]	1.55	1.38	1.21	0.026	<0.01	<0.01	0.97	<0.01	0.75
C20:4	0.59	0.58	0.55	0.011	<0.01	<0.01	1.00	<0.01	0.46
Others[4]	18.28	16.60	14.80	0.314	<0.01	<0.01	0.99	<0.01	0.69
Total	245.06	271.99	291.33	5.084	<0.01	<0.01	0.97	<0.01	0.18

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Significance of effects for treatment (**Trt**), week (**wk**), treatment × week (**Trt × wk**), and linear (**L**) and quadratic (**Q**) orthogonal contrasts.

[3] Includes all C18:2 *trans* isomers.

[4] Sum of C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C11:0, C11:1, C13:0, C13:1, C14:1, C15:0, C15:1, C16:1 *trans*, C17:0, C17:1, C18:1, *trans* 6, C18:1, *trans* 9, C18:1, *trans* 10, C18:1, *cis* 9, C20:1, 5, C20:1, 8, C20:1 *cis*, C18:2, *trans* 10, *cis* 12, C18:2, *cis* 9, *trans* 11, C20:2, 11, 14, C20:3 *homo* ɤ, C22:0, C20:3, 11, 14, 17, C22:1, C23:0, C20:5, C22:2, C24:0, C22:3, C22:4, C24:1, C22:5, N3, C22:6, and unidentified fatty acids.

Table 8. Dry matter intake, body weights, and gain to feed ratios for Holstein heifers fed increasing inclusion amounts of RFDDGS in replacement of grass hay.

	Treatment[1]				*P-values*[2]				
Item	30DG	40DG	50DG	SEM	Trt	wk	Trt × wk	L	Q
Age, initial	198.1 ± 1.93	200.3 ± 1.93	199.2 ± 1.93		0.49				
BW, kg									
Mean	264.1	266.2	266.4	4.98	0.69	<0.01	1.00	0.44	0.72
Initial	206.6	205.1	206.1	1.95	0.85				
Final	307.6	312.5	313.0	7.51					
ADG[3], kg/d	0.89 ±0.071	0.94 ± 0.083	0.97 ± 0.083		0.43				
DMI, kg									
Mean	6.49	6.21	5.84	0.117	<0.01	<0.01	1.00	<0.01	0.50
Final	7.75	7.37	7.05	0.178					
Gain:Feed									
Mean	0.142	0.161	0.183	0.0034	<0.01	<0.01	0.94	<0.01	0.02
Final	0.119	0.130	0.145	0.0057					

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).
[2]Significance of effects for treatment (**Trt**), week (**wk**), treatment × week (**Trt × wk**), and linear (**L**) and quadratic (**Q**) orthogonal contrasts.
[3]Calculated using regression analysis of BW over the d of the study.

Table 9. Frame size measurements for Holstein heifers fed treatment diets with increasing inclusion amounts of RFDDGS in replacement of grass hay.

	Treatments[1]				*P- values*[2]				
Item	30DG	40DG	50DG	SEM	Trt	wk	Trt × wk	L	Q
Withers height, cm									
Mean	121.0	121.7	121.6	0.29	<0.01	<0.01	0.99	<0.01	0.03
Initial	113.5	113.1	114.5	0.32	<0.01				
Final	127.5	127.1	127.1	0.50					
Change[3], cm/d	0.114 ±0.009	0.118 ±0.009	0.115 ±0.011	-	0.93				
Hip height, cm									
Mean	124.8	124.7	124.8	0.36	0.74	<0.01	1.00	0.98	0.44
Initial	115.3	116.2	117.3	0.51	<0.01				
Final	130.0	130.1	130.2	0.58					
Change[3], cm/d	0.117 ±0.009	0.116 ±0.009	0.113 ±0.011	-	0.78				
Heart girth, cm									
Mean	140.9	140.6	141.0	0.41	0.22	<0.01	0.95	0.81	0.08
Initial	130.9	131.2	130.7	0.79	0.76				
Final	149.1	148.9	149.7	0.62					
Change[3], cm/d	0.171 ±0.014	0.170 ±0.018	0.181 ±0.015	-	0.65				
Paunch girth, cm									
Mean	172.5	173.9	172.5	1.29	0.02	<0.01	0.99	0.98	<0.01
Initial	163.7	162.0	162.1	1.02	0.16				
Final	179.9	182.9	180.8	1.70					
Change[3], cm/d	0.173 ±0.021	0.199 ±0.025	0.201 ±0.019	-	0.37				
Body length, cm									
Mean	112.5	112.9	113.1	0.64	0.18	<0.01	1.00	0.06	0.85
Initial	101.0	101.6	101.5	0.44	0.30				
Final	118.0	119.0	118.7	0.95					
Change[3], cm/d	0.116 ±0.009	0.123 ±0.011	0.123 ±0.010	-	0.63				
Hip width, cm									
Mean	35.62	35.82	35.76	0.317	0.57	<0.01	1.00	0.46	0.45
Initial	31.19	32.11	32.43	0.153	0.30				
Final	38.18	38.50	38.42	0.612					
Change[3], cm/d	0.054 ±0.005	0.058 ±0.006	0.058 ±0.005	-	0.58				
BCS[4]									
Mean	3.11	3.12	3.07	0.018	<0.01	0.59	0.82	<0.01	0.02
Initial	3.17	3.19	3.15	0.018	0.06				
Final	3.08	3.11	3.08	0.035					

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Significance of effects for treatment (**Trt**), week (**wk**), treatment × week (**Trt × wk**), and linear (**L**) and quadratic (**Q**) orthogonal contrasts.

[3]Calculated using regression analysis of body measurement over the d of the study.

[4] Body condition score with 1 = emaciated and 5 = obese (Wildman et al., 1982).

Table 10. Rumen fermentation parameters of Holstein heifers fed increasing amounts of RFDDGS in replacement of forage

	Treatments[1]				*P-values*[2]				
Item	30DG	40DG	50DG	SEM	Trt	wk	Trt × wk	L	Q
pH	6.67	6.55	6.52	0.084	0.03	0.10	0.47	0.02	0.32
NH_3-N, mg/dL	15.4	17.0	19.3	0.850	<0.01	0.61	0.37	<0.01	0.71
Acetate, m*M*	43.4	41.8	41.7	1.41	0.41	0.30	0.06	0.23	0.56
Propionate, m*M*	18.1	19.9	22.6	0.92	<0.01	0.02	0.21	<0.01	0.53
Isobutyrate, m*M*	0.87	0.95	0.95	0.038	0.08	0.34	0.03	0.06	0.23
Butyrate, m*M*	8.88	8.54	7.26	0.364	<0.01	0.29	0.25	<0.01	0.17
Isovalerate, m*M*	0.48	0.57	0.50	0.030	0.04	0.19	0.51	0.70	0.01
Valerate, m*M*	1.33	1.30	1.24	0.053	0.44	0.07	0.05	0.21	0.81
Total VFA, m*M*	73.1	73.1	74.2	2.45	0.86	0.10	0.07	0.63	0.79
Acetate, m*M*/100m*M*	59.4	57.4	56.2	0.476	<0.01	0.06	0.37	<0.01	0.31
Propionate, m*M*/100m*M*	24.7	27.0	30.4	0.60	<0.01	0.16	0.18	<0.01	0.27
Isobutyrate, m*M*/100m*M*	1.20	1.31	1.28	0.042	0.01	0.56	0.26	0.03	0.03
Butyrate, m*M*/100m*M*	12.2	11.8	9.8	0.42	<0.01	0.79	0.48	<0.01	0.02
Isovalerate, m*M*/100m*M*	0.67	0.80	0.68	0.039	0.03	0.03	0.37	0.78	<0.01
Valerate, m*M*/100m*M*	1.80	1.78	1.67	0.054	0.11	0.15	0.23	0.05	0.42
Acetate:Propionate	2.44	2.18	1.90	0.054	<0.01	0.04	0.18	<0.01	0.79

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Significance of effects for treatment (**Trt**), week (**wk**), treatment × week (**Trt × wk**), and linear (**L**) and quadratic (**Q**) orthogonal contrasts.

Table 11. Total tract digestibility of nutrients for Holstein heifers fed increasing amounts of RFDDGS in replacement of grass hay.

	Treatments[1]				*P-values*[2]		
Item, %	30DG	40DG	50DG	SEM	Trt	L	Q
DM	64.7	68.3	72.9	1.92	<0.01	<0.01	0.71
OM	66.4	69.8	74.0	1.92	<0.01	<0.01	0.77
CP	73.7	79.5	86.0	1.90	<0.01	<0.01	0.80
NDF	54.6	57.1	58.6	3.75	0.27	0.11	0.82
ADF	50.8	52.4	53.4	2.17	0.69	0.39	0.90

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).
[2]Significance of effects for treatment (**Trt**) and linear (**L**) and quadratic (**Q**) orthogonal contrasts.

Table 12. Nutrient intake and estimated fecal output during the three day collection period for total tract digestibility determination for Holstein heifers fed increasing amounts of RFDDGS in replacement of grass hay in limit-fed rations.

	Treatments[1]				*P-values*[2]		
Item, g/d	30DG	40DG	50DG	SEM	Trt	L	Q
DM intake	7,793	7,398	6,995	190.9	0.01	<0.01	0.99
DM output	4,926	4,950	5,025	249.3	0.89	0.65	0.89
OM intake	7,110	6,771	6,431	175.6	0.03	<0.01	1.00
OM output	4,241	4,250	4,342	201.5	0.81	0.57	0.78
CP intake	1,334	1,450	1,538	67.61	<0.01	<0.01	0.75
CP output	644.8	674.8	695.1	24.27	0.33	0.14	0.86
N input	213.5	232.0	246.0	10.82	<0.01	<0.01	0.75
N output	103.2	108.0	111.2	3.88	0.33	0.14	0.86
NDF intake	4,246	3,772	3,220	102.7	<0.01	<0.01	0.92
NDF output	3,259	3,201	3,277	150.8	0.84	0.89	0.57

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Significance of effects for treatment (**Trt)** and linear (**L**) and quadratic (**Q**) orthogonal contrasts.

Table 13. Plasma fatty acid profile from wk 16 of the feeding period for Holstein heifers fed increasing amounts of RFDDGS in replacement of grass hay in limit-fed rations.

	Treatments[1]				*P-values*[2]		
Item[3], mg/100 mg fatty acid	30DG	40DG	50DG	SEM	Trt	L	Q
C4:0	0.98	0.99	0.90	0.033	0.12	0.09	0.22
C5:0	3.09	3.22	2.81	0.123	0.06	0.12	0.08
C6:0	0.08	0.09	0.08	0.013	0.72	0.82	0.44
C7:0	0.06	0.08	0.06	0.010	0.26	0.98	0.10
C13:0	0.08	0.07	0.08	0.018	0.99	0.97	0.87
C14:0	0.81	0.80	0.65	0.073	0.02	0.01	0.19
C14:1	0.43	0.43	0.37	0.032	0.02	0.01	0.23
C15:0	0.71	0.68	0.64	0.031	0.04	0.01	0.68
C15:1	0.26	0.27	0.24	0.069	0.82	0.70	0.62
C16:0	11.80	12.11	11.77	0.250	0.25	0.88	0.10
C16:1 *trans*	0.85	0.99	0.94	0.114	0.15	0.22	0.13
C16:1 *cis*	0.77	0.81	0.77	0.031	0.55	0.98	0.28
C17:0	0.92	0.84	0.82	0.068	0.20	0.09	0.60
C17:1	0.17	0.14	0.11	0.017	0.07	0.02	0.95
C18:0	18.54	19.38	18.91	0.343	0.23	0.45	0.12
C18:1, *trans* 6	0.57	0.63	0.51	0.045	0.02	0.12	0.01
C18:1 *trans* 10	1.54	1.66	1.37	0.139	0.09	0.19	0.08
C18:1*cis 9*	7.20	7.44	6.58	0.260	0.06	0.10	0.09
C18:1 *cis* 11	0.52	0.48	0.42	0.050	0.02	<0.01	0.75
C18:1 *trans* 11	0.09	0.08	0.07	0.015	0.57	0.30	0.86
C18:2, *cis* 9, *cis* 12	36.47	35.40	38.65	0.817	0.02	0.07	0.04
C18:3 ɣ	2.23	2.45	2.13	0.141	0.28	0.62	0.13
C18:3 α	2.56	1.97	1.72	0.264	<0.01	<0.01	0.36
C19:0	0.17	0.18	0.15	0.017	0.58	0.64	0.36
C20:0	0.09	0.12	0.10	0.009	0.06	0.46	0.02
C20:1 *cis*	0.09	0.10	0.09	0.025	0.81	0.91	0.52
C20:2, 11, 14	0.09	0.10	0.10	0.016	0.76	0.57	0.63
C20:3 *homo* ɣ	2.34	2.43	2.44	0.093	0.68	0.42	0.75
C20:4	3.98	3.82	4.21	0.200	0.39	0.43	0.27
C20:5	0.21	0.18	0.15	0.018	0.14	0.05	0.95
C22:4	0.41	0.40	0.53	0.067	0.03	0.03	0.13
C24:0	0.12	0.10	0.11	0.012	0.69	0.75	0.43
C24:1	0.17	0.16	0.21	0.057	0.48	0.41	0.38
C22:5, *N*3	0.82	0.69	0.67	0.051	0.08	0.04	0.37
C22:6	0.14	0.11	0.06	0.037	0.28	0.11	0.83
Others[4]	0.10	0.04	0.01	0.030	0.11	0.04	0.66
> C16:0	81.88	81.50	82.68	0.290	0.02	0.06	0.03
< C16:0	6.57	6.71	5.90	0.478	0.02	0.02	0.06
MUFA	12.55	13.08	11.58	0.619	0.02	0.06	0.03
PUFA	49.50	47.80	50.92	0.722	0.01	0.17	0.01

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Significance of effects for treatment (**Trt**) and linear (**L**) and quadratic (**Q**) orthogonal contrasts.

[3] Represented as number of carbons: number of double bonds.

[4] Sum of C8:0, C9:0, C10:0, C11:0, C12:0, C12:1, C18:1 *trans* 9, C20:1, 5, C20:1, 8, C18:2 *trans* 9, *trans* 10, 11, 12, C18:2 *cis* 9, *trans* 11, C18:2 *trans* 10, *cis* 12, C18:2 *cis* 10, 12, C22:0, C22:3 11, 14, 17, C22:1, C22:2, C22:3, and unidentified Fatty Acids.

Table 14. Plasma fatty acid concentrations from wk 16 of the feeding period for Holstein heifers fed increasing amounts of RFDDGS in replacement of grass hay in limit-fed rations.

	Treatments[1]				*P-values*[2]		
Item[3], µg/mL plasma	30DG	40DG	50DG	SEM	Trt	L	Q
C4:0	13.18	13.17	13.38	0.075	0.10	0.08	0.22
C5:0	41.57	42.86	41.87	0.933	0.60	0.82	0.32
C6:0	1.13	1.25	1.18	0.186	0.90	0.84	0.69
C7:0	0.74	0.99	0.84	0.110	0.27	0.51	0.14
C13:0	1.11	1.13	1.17	0.240	0.95	0.76	0.95
C14:0	11.22	10.87	10.12	0.885	0.27	0.12	0.74
C14:1	6.29	6.11	5.92	0.303	0.69	0.39	0.99
C15:0	9.96	9.56	10.03	0.349	0.59	0.89	0.31
C15:1	3.72	3.88	3.80	0.784	0.96	0.88	0.81
C16:0	163.92	166.81	181.25	9.075	0.05	0.02	0.37
C16:1 *trans*	11.64	13.54	14.40	0.788	0.05	0.02	0.60
C16:1 *cis*	10.36	11.00	11.57	0.531	0.28	0.11	0.96
C17:0	13.21	11.96	13.09	1.381	0.44	0.91	0.20
C17:1	2.29	1.89	1.66	0.245	0.20	0.08	0.79
C18:0	257.16	266.75	291.81	23.356	0.08	0.03	0.56
C18:1 *trans* 6	7.97	8.80	8.14	0.713	0.41	0.80	0.19
C18:1 *trans* 10	21.60	22.98	22.13	2.307	0.79	0.80	0.53
C18:1 *cis 9*	98.25	100.15	99.95	4.797	0.95	0.80	0.86
C18:1 *cis* 11	7.12	6.54	6.63	0.385	0.46	0.28	0.55
C18:1 *trans* 11	1.19	1.10	1.18	0.240	0.90	0.93	0.65
C18:2, *cis* 9, *cis* 12	495.44	483.83	589.02	22.515	<0.01	<0.01	0.04
C18:3 ɤ	32.13	34.80	34.15	4.519	0.69	0.53	0.56
C18:3 α	36.52	28.07	28.12	4.854	0.02	0.02	0.15
C19:0	2.27	2.44	2.35	0.260	0.90	0.82	0.68
C20:0	1.21	1.60	1.47	0.136	0.13	0.19	0.13
C20:1 *cis*	1.23	1.37	1.44	0.378	0.78	0.49	0.90
C20:2, 11, 14	1.22	1.43	1.57	0.228	0.54	0.28	0.89
C20:3 *homo* ɤ	31.70	33.28	37.25	1.837	0.10	0.04	0.60
C20:4	55.57	52.12	65.34	6.653	0.01	0.03	0.04
C20:5	2.84	2.38	2.32	0.263	0.32	0.17	0.54
C22:4	5.79	5.39	8.24	0.999	<0.01	<0.01	0.02
C24:0	1.63	1.42	1.71	0.169	0.46	0.75	0.23
C24:1	2.58	2.28	3.27	0.849	0.36	0.34	0.29
C22:5, *N*3	11.25	9.17	10.05	0.751	0.16	0.27	0.11
C22:6	2.07	1.41	0.87	0.490	0.23	0.09	0.92
Others[4]	1.40	0.56	0.21	0.409	0.12	0.05	0.63
Total	1,361.22	1,355.60	1,520.14	45.780	0.02	0.02	0.14
> C16:0	1,115.21	1,106.64	1,258.59	40.487	0.02	0.02	0.11
< C16:0	88.82	89.71	88.22	1.437	0.76	0.77	0.50
MUFA	179.24	184.62	184.99	7.409	0.83	0.59	0.78
PUFA	673.29	650.63	775.69	26.946	<0.01	0.01	0.03

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Significance of effects for treatment (**Trt**) and linear (**L**) and quadratic (**Q**) orthogonal contrasts.

[3] Represented as number of carbons: number of double bonds.

[4] Sum of C8:0, C9:0, C10:0, C11:0, C12:0, C12:1, C18:1 *trans* 9, C20:1, 5, C20:1, 8, C18:2 *trans* 9, *trans* 10, 11, 12, C18:2 *cis* 9, *trans* 11, C18:2 *trans* 10, *cis* 12, C18:2 *cis* 10, 12, C22:0, C22:3 11, 14, 17, C22:1, C22:2, C22:3, and unidentified Fatty Acids.

Table 15. Plasma metabolites and metabolic hormone concentrations for Holstein heifers fed the treatment diets with increasing amounts of RFDDGS in replacement of grass hay in limit-fed rations.

	Treatments[1]				*P values*[2]				
Item	30DG	40DG	50DG	SEM	Trt	Wk	Trt× Wk	L	Q
Cholesterol, mg/dL	93.48	89.15	97.13	2.155	<0.01	<0.01	0.74	0.14	<0.01
Glucose[3], mg/dL	76.26	77.74	77.33	1.601	0.41	0.13	0.91	0.35	0.34
IGF-1, ng/mL	102.70	99.98	109.38	3.595	0.03	<0.01	0.51	0.06	0.05
Insulin, ng/mL	1.04	1.13	1.15	0.093	0.36	<0.01	0.86	0.19	0.59
Leptin, ng/mL	4.42	4.35	4.59	0.087	0.13	0.15	0.59	0.17	0.14
PUN, mg/dL	17.83	17.82	19.90	0.350	<0.01	<0.01	0.96	<0.01	0.01
Triglycerides, mg/dL	17.82	19.14	18.47	0.699	0.41	0.88	0.51	0.51	0.24

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

[2]Significance of effects for treatment (**Trt**), week (**wk**), treatment × week (**Trt × wk**), and linear (**L**) and quadratic (**Q**) orthogonal contrasts.

[3]Glucose was measured from serum samples instead of plasma.

Table 16. Mean age and body weight at puberty for Holstein heifers fed increasing inclusion amounts of reduced-fat distillers dried grains with solubles (**RFDDGS**) in replacement of forage limit-fed rations.

	Treatment[1]				*P-value*
Item	30DG	40DG	50DG	SEM	Trt
Age at puberty, d	234.6	244.3	235.5	13.7	0.80
Body weight at puberty, kg	246.4	261.3	254.0	24.9	0.59

[1]30% dietary inclusion rate of RFDDGS (**30DG**); 40% dietary inclusion rate of RFDDGS (**40DG**); 50% dietary inclusion rate of RFDDGS (**50DG**).

Figure 1. Percentage of Holstein heifers pubertal (cycling) by age that were fed increasing amounts of RFDDGS in replacement of grass hay in limit-fed rations.

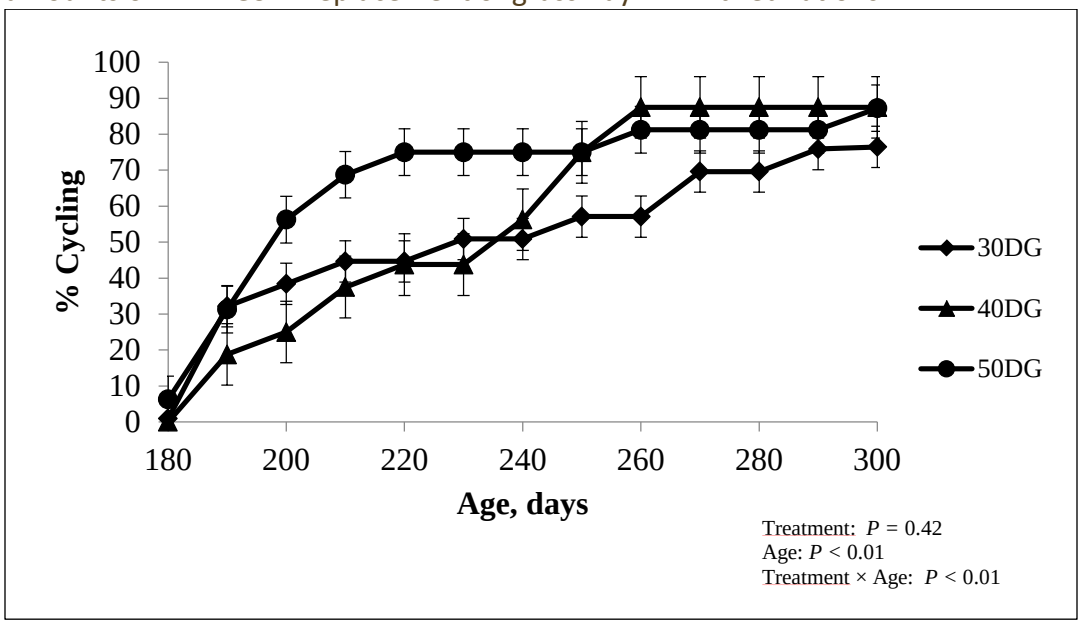

Figure 2. Percent of Holstein heifers pubertal (cycling) by body weight that were fed increasing amounts of RFDDGS in replacement of grass hay in limit-fed rations.