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Growth Performance, Nutrient Utilization, and Metabolic Profile of Dairy Heifers Fed Diets High in Distillers Grains with Different Forage to Concentrate Ratios

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GROWTH PERFORMANCE, NUTRIENT UTILIZATION, AND METABOLIC PROFILE OF DAIRY HEIFERS FED DIETS HIGH IN DISTILLERS GRAINS WITH DIFFERENT FORAGE TO CONCENTRATE RATIOS

BY

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2016

GROWTH PERFORMANCE, NUTRIENT UTILIZATION, AND METABOLIC PROFILE OF DAIRY HEIFERS FED DIETS HIGH IN DISTILLERS GRAINS WITH DIFFERENT FORAGE TO CONCENTRATE RATIOS

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
ADG	Average daily gain
ADIA	Acid detergent insoluble ash
AI	Artificial insemination
BCS	Body condition score
BW	Body weight
Ca	Calcium
Cl	Chloride
CL	Corpus luteum
cm	centimeter
CP	Crude protein
CV	Coefficient of variation
dL	deciliter
DDG	Distillers dried grains with solubles concentrate treatment
DDGS	Distillers dried grains with solubles
DHIA	Dairy Herd Improvement Association
DM	Dry matter
DMI	Dry matter intake
DIM	Days in milk
DWGS	Distillers wet grains with solubles
EE	Ether extract
FA	Fatty acid

Fe	Iron
GnRH	Gonadotropin releasing hormone
IGF-1	Insulin-like growth factor -1
K	Potassium
kg	kilogram
K_2EDTA	potassium ethylene diamine tetra-acetic acid
L	Linear
LH	Luteinizing hormone
Mcal	Mega calories
ME	Metabolizable energy
Mg	Magnesium
mg	milligram
mL	milliliter
Mn	Manganese
Mo	Molybdenum
µg	micrograms
N	Nitrogen
Na	Sodium
NaFl	Sodium fluoride
NDF	Neutral detergent fiber
NFC	Non-fibrous carbohydrate
ng	nanograms
NE_g	Net energy gain

NH_3	Ammonia
OM	Organic matter
P	Phosphorus
PUFA	Polyunsaturated fatty acid
PUN	Plasma urea nitrogen
Q	Quadratic
RDP	Rumen degradable protein
RIA	Radioimmunoassay
RUP	Rumen undegradable protein
S	Sulfur
SBM	Soybean Meal
SCC	Somatic cell count
SEM	Standard error of the mean
TMR	Total mixed ration
trt	treatment
VFA	Volatile fatty acid
wk	week
Zn	Zinc

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ABSTRACT

GROWTH PERFORMANCE, NUTRIENT UTILIZATION, AND METABOLIC PROFILE OF DAIRY HEIFERS FED DIETS HIGH IN DISTILLERS GRAINS WITH DIFFERENT FORAGE TO CONCENTRATE RATIOS

ANGELA KRISTIA MANTHEY

2016

Two studies were conducted to evaluate the effect of limit-feeding heifers distillers grains with solubles (**DDGS**) with varying forage to concentrate ratios. The effects on growth, feed efficiency, rumen fermentation, nutrient digestibility, metabolic profile and onset of puberty, as well as post trial performance were investigated. First, a 16-wk feeding trial was conducted using 48 Holstein heifers to evaluate effects of dietary treatment on dry matter intake (**DMI**), average daily gain (**ADG**), growth performance, rumen fermentation, and nutrient digestibility. Treatments were 1) 30% DDGS, with the diet fed at 2.65% of body weight (**BW**) (**30DG**), 2) 40% DDGS, with the diet fed at 2.50 % of BW (**40DG**), and 3) 50% DDGS, with the diet fed at 2.35% of BW (**50DG**). The remainder of the diet consisted of grass hay and 1.5% mineral mix. Heifers were individually limit-fed using Calan gates. There were no differences in growth parameters; however, gain: feed and nutrient digestibility increased with increasing amounts of DDGS. There was a linear increase in concentration of plasma linoleic acid with increasing amount of DDGS and a linear and quadratic response for arachidonic acid. Total fatty acid **(FA)** and polyunsaturated fatty acids (**PUFA**) were linearly increased with a quadratic response with 30DG and 50DG having the greatest concentrations. There was a quadratic response of plasma urea nitrogen (**PUN**) and a quadratic tendency for cholesterol. Age and BW at puberty were similar among treatments. After heifers completed the feeding trial, data were collected to assess post trial reproductive and lactation performance, which were comparable among treatments. . A second study was conducted to determine the effects of feeding a corn and soybean product based concentrate mix or distillers dried grains with solubles (**DDGS**) concentrate mix with ad libitum grass hay to dairy heifers. A 16-wk feeding trial was conducted using 24 heifers to evaluate the effect of diet on DMI, growth performance, rumen fermentation, metabolic profile, and nutrient digestibility. Treatments were 1) corn and soybean product concentrate mix (**CON**), and 2) DDGS based concentrate mix (**DDG**). Both concentrate mixes were limit-fed at 0.8% of BW and grass hay was offered ad libitum. Dry matter intake and growth parameters did not differ between treatments. Rumen fermentation was shifted, but metabolic profile was maintained for heifers fed DDG. Results from these studies indicate that the fat and protein in DDGS can be used as a replacement for the starch in corn in limit-fed heifer diets with varying forage to concentrate ratios to maintain growth performance, nutrient digestibility, and metabolic profile without detrimental effects to long-term performance.

INTRODUCTION

Dairy heifer nutrition has been an increasing area of interest in research, as plane of nutrition of the heifer can impact future production performance. To manipulate the plane of nutrition various rearing strategies have been investigated. Over the years researchers have been trying to determine the optimal rate of gain at which heifers should be raised and various feedstuffs have been examined as viable options.

In recent years, limit-feeding heifers has been increasingly researched. Limit-feeding limits the caloric intake of the heifers by feeding a nutrient dense diet, decreasing the amount of feed that is fed. It has proven to be successful at increasing gain: feed while also increasing nutrient digestibility and decreasing the amount of feed that is wasted (Hoffman et al., 2007; Zanton and Heinrichs, 2009; Anderson et al., 2015a). However, research in which the limit-feeding strategy has been implemented has usually been done with corn and soybean product based diets. There has been limited research in which alternative feedstuffs, like distillers grains, have been investigated.

Due to the increased development of the ethanol industry within the Midwest, distillers grains have become readily available as an alternative feedstuff. Feeding distillers grains has been well investigated in beef and lactating dairy cattle (Anderson et al., 2006; Martin et al., 2007; Klopfenstein et al., 2008; Schingoethe et al., 2009). However, there is very limited research in which distillers grains has been investigated in dairy heifer diets (Anderson et al., 2009; Schroer et al., 2014; Anderson 2015a, b, c). When distillers grains were fed to dairy heifers in research studies, diets with high forage concentrations have typically been utilized.

Therefore, this research will focus upon examining the effect of increasing the inclusion amount of DDGS in limit-fed dairy heifer diets with different forage to concentrate ratios. It will also investigate feeding DDGS with ad libitum grass hay. The overall objective is to determine the optimal forage to concentrate ratio in which to feed DDGS in limit-fed rations to optimize growth performance, gain: feed, and nutrient digestibility. Overall the hypothesis is that replacing the starch from corn with the fat from distillers grains in limit-fed dairy heifer diets will maintain heifer growth performance and increase feed efficiency and nutrient digestibility.

CHAPTER 1:

LITERATURE REVIEW

In the Midwestern United States, the ethanol industry has provided opportunities for dairy nutritionists and producers to explore the use of distillers grains in dairy heifer diets. The alternative feedstuff is readily available and economically attractive making it a viable alternative protein and energy source. The higher fat content of distillers grains with solubles (**DDGS**) compared to traditional feed ingredients such as corn and soybean meal has made it difficult to incorporate into dairy heifer diets at high inclusion amounts. The new development of reduced-fat DDGS, in which some of the fat has been removed through centrifugation, may allow for its incorporation into the diet at much greater proportions. Producers could then utilize this ethanol co-product in dairy heifer rations, making it a suitable replacement for other feedstuffs. Distillers grains are also more economical to ship long distances compared to forages, making feeding DDGS useful in areas of the United States where forages are limited. However, there are still many research opportunities in which to further understand how the ethanol co-product affects heifer growth and development, nutrient utilization, and long-term reproductive and lactation performance.

Raising Replacement Heifers

Goals and Challenges

Replacement heifers represent the future potential of a dairy operation; therefore, great attention must be paid to heifer rearing programs. The goal of a dairy heifer replacement program is to raise heifers at a low economic and environmental cost without compromising future production potential, health, or welfare (Sejrsen and Purup, 1997; Hoffman et al., 1997; Kitts et al., 2011). This is especially important, as raising replacement heifers accounts for the second greatest expense on the dairy operation, second only to the feed costs of the lactating herd (Heinrichs, 1993). Replacement heifers also provide the producer no immediate financial benefits until calving and the onset of lactation (Kitts et al., 2011).

First calving and the onset of lactation usually occur when heifers are 22-24 months of age (Ettema and Santos, 2004). It is not until then that return is finally made on investments, so any improvement on efficiency is of value (Heinrichs, 1993). A balance between getting the heifer to reproductive age in a timely manner without allowing a high rate of gain must be met. It is thought that the heifer will be at risk for metabolic disorders such as acidosis and laminitis and poor mammary development if the rate of gain is too high (Abeni et al., 2000; Zanton and Heinrichs, 2005). This is a concern because replacement heifers are needed to replace approximately 40% of the lactating herd each year (Kitts et al., 2011).

Recommendations for Average Daily Gain

One strategy to reduce the costs associated with raising heifers involves reducing the length of the growing period. To do so, prepubertal average daily gain (**ADG**) is increased in an effort to decrease age at first calving. However, this strategy could potentially decrease future lactation potential (Zanton and Heinrichs, 2005).

There may be reduced mammary development due to increased prepubertal ADG (Sejrsen et al., 1982). The mammary gland grows at an allometric rate prior to puberty and then an isometric rate after the onset of puberty (Sinha and Tucker, 1969). This may be explained by insulin-like growth factor -1 (**IGF-1**) receptors becoming less sensitive when high energy diets are fed because of reduced concentrations of circulating growth hormone as a result of negative feedback from IGF-1 (Sejrsen and Purup, 1997). Meyer et al. (2006b) fed heifers an elevated or restricted level of nutrients to support 950 or 650 g/d of ADG and investigated the effect on mammary development. It was demonstrated that elevated nutrient intake during the prepubertal period did not influence mammary epithelial cell proliferation (Meyer et al., 2006b). Despite treatments, there was a 50% reduction in mammary parenchyma DNA accretion when heifers were between 250 and 300 kg of BW demonstrating that the mammary gland was transitioning from allometric to isometric growth (Meyer et al., 2006b).

Van Amburgh et al. (1998) fed heifers from 90 to 320 kg one of three diets that were designed to achieve ADG of 0.6, 0.8, and 1.0 kg/d, and protein source also varied within each energy treatment. Actual ADG for each energy treatment were 0.68, 0.83, and 0.94 kg/d. There were no differences in ADG or milk yield due to protein source. However, milk yield was decreased 5% for heifers grown at an excess of 0.7 kg/d during the prepubertal period (Van Amburgh et al., 1998). Furthermore, heifers that had reached a body weight (**BW**) that was 82 - 90% of mature size at calving had greater first lactation milk yield (Van Amburgh et al., 1998). It was also concluded that protein supplementation may have met the requirements of the tissue to increase gain, along with adequate energy, enabling heifers to reach breeding at an earlier weight without having detrimental effects on mammary development (Van Amburgh et al., 1998).

Zanton and Heinrichs (2005) conducted a meta-analysis to determine the effects that prepubertal ADG of Holstein heifers had on milk production, fat corrected milk yield, milk fat, and milk protein in the first lactation. It was demonstrated that for heifers between 150 and 320 kg gaining 0.8 kg/d, maximized milk and protein yield in the first lactation (Zanton and Heinrichs, 2005). This research indicated that ADG should be restricted to 0.8 kg/d to avoid negative effects on lactation potential.

However, Anderson et al. (2015c) limit-fed prepubertal dairy heifers a control, low-fat DDGS, or high-fat DDGS diet and achieved ADG of 0.95, 0.96, and 0.98 kg/d, respectively, indicating that heifers were on a high plane of nutrition. Rate of gain for these heifers was controlled by utilizing a limit-feeding strategy, but was still greater than the recommended 0.8 kg/d ADG. Feeding DDGS maintained or increased milk production in these heifers, indicating that form of energy (starch versus fat) may play a role in future production (Anderson et al., 2015c).

Forage to Concentrate Ratio

Heifers are traditionally fed diets with greater forage components. However, increasing the inclusion of high fiber components of the diet may decrease diet digestibility, as well as result in an energy and protein inefficiency (Moody et al., 2007; Zanton and Heinrichs, 2007; Zanton and Heinrichs, 2008). Zanton and Heinrichs (2008) found that as dairy heifers were fed high forage diets at intakes needed for maintenance or ad libitum, the efficiency of nutrient utilization was increased as intake decreased.

Additionally, beef heifers that were fed a constant metabolizable energy (**ME**) from a high-concentrate (25: 75) or low-concentrate (75: 25) had less heat energy production and retained more tissue energy when fed the high-concentrate diet (Reynolds et al., 1991). This suggests that high-concentrate diets could be used to reduce the dry matter intake (**DMI**) of the animal, while still meeting the nutrient requirements (Zanton and Heinrichs, 2007). However, feeding high-concentrate diet intakes may need to be restricted in order to avoid increased ADG. Feeding high-concentrate diets has resulted in decreased manure output, similar milk yields when ADG was controlled, and had no negative effect on rumen fermentation (Hoffman et al., 2007; Moody et al., 2007; Zanton and Heinrichs, 2007; Lascano and Heinrichs, 2009). However, there may be differences in nitrogen (**N**) partitioning and utilization when different forage concentrations are fed (Zanton and Heinrichs, 2009).

Limit-Feeding Strategy

Limit-feeding is not a new feeding strategy. Over the years, research has shown that it has proven successful in beef cows, beef heifers, ewes, and beef steers (Loerch, 1990; Susin et al., 1995; Loerch, 1996; Wertz et al., 2001). Wertz et al. (2001) conducted a trial utilizing 140 crossbred beef heifers. The trial evaluated intake restriction on the performance and carcass merit of heifers limit-fed or ad-libitum fed corn gluten feed (Wertz et al., 2001). During the finishing phase, limit-fed beef heifers did not have compromised feed efficiency compared to ad-libitum fed heifers. Gain to feed was 0.124 vs. 0.135 gain: feed, kg/kg, ad-libitum and limit-fed, respectively. Also, diets allowed all heifers to achieve a moderate rate of gain (Wertz et al., 2001). Overall, the combined growing-finishing gain to feed ratio was also similar when comparing heifers that were limit-fed and heifers that were ad-libitum fed 0.157 vs. 0.167 kg/kg, respectively.

Previous research has demonstrated that limit-feeding dairy heifers does not negatively impact growth characteristics. Zanton and Heinrichs (2007) investigated the effect of feeding high forage or high concentrate rations for similar rates of prepubertal ADG. Forty-two heifers that were approximately 4 months of age were assigned to either a high forage or a high concentrate ration and were individually fed using Calan doors. Diets were formulated using grass and corn silages and were limit-fed to achieve 0.8 kg/d ADG. Dietary treatments caused no differences in BW gain, withers height, heart girth, body length, or hip width (Zanton and Heinrichs, 2007). However, heifers fed the high forage diet initially had a greater paunch girth when compared to those fed the high concentrate diet at 149 and 141 cm, respectively. The daily paunch girth gain during the study was less for the heifers fed high forage diet compared to those fed the high concentrate diet (0.190 and 0.247 cm/d, respectively; Zanton and Heinrichs, 2007). As a result of gut fill from eating more fibrous material, heifers fed the high forage diet were expected to maintain a greater paunch girth gain. Therefore, greater paunch girth gain in the high concentrate fed heifers would have to be a result of a differing composition of the paunch. The study did not measure heifer body composition, but the high concentrate diet was not expected to result in greater visceral fat.

It has also been demonstrated that heifers that are limit-fed do not have decreased lactation performance. Zanton and Heinrichs (2007) investigated the effect of limit-feeding prepubertal heifers a high forage or a high concentrate ration, and its subsequent effect on lactation. Milk yield tended to decrease in heifers limit-fed a high forage diet compared to a high concentrate diet, with 8,740 and 9,776 kg projected for the first 305d lactation, respectively (Zanton and Heinrichs, 2007). Fat yield was also decreased in heifers limit-fed a high forage diet compared to a high concentrate, 323 and 385 kg projected for the first 305 d lactation, respectively (Zanton and Heinrichs, 2007). Hoffman et al. (2007) conducted research in which gravid heifers were limit-fed and subsequent lactation performance was investigated. There were no differences in milk yield, milk fat yield, milk fat percentage, milk protein percentage, or milk protein yield (Hoffman et al., 2007). Therefore, limit-fed heifers do not have decreased lactation performance, and may actually have improved milk and fat yield based on the projected first 305 d lactation data.

Slowed rate of passage caused by limit-feeding results in greater ruminal retention time and increased ruminal degradation and utilization of nutrients. Loerch (1990) conducted a study to determine the effects of limit-feeding high-energy diets on beef cattle performance and diet digestibility. One hundred twenty Angus or Angus crossbred steers (246 kg) were pen fed one of three dietary treatments: a corn silage based diet fed ad-libitum, a whole-shelled, high-moisture corn, corn silage based diet limit-fed 20% below ad-libitum, and a whole-shelled, high-moisture corn, corn silage based diet limit-fed 30% below ad-libitum (Loerch, 1990). Diet digestibility percentage decreased in the ad-libitum fed steers compared to those that were limit-fed to 20 and 30% of ad-libitum, 65.0, 72.0, and 88.6%, respectively (Loerch, 1990). Tamminga et al. (1979) also conducted a study investigating the effect of the level of feed intake on the quantity of protein entering the small intestine. The degradation of dietary protein within the forestomach was estimated using two methods, one based upon diaminopimelic acid as a marker and the other based upon regression (Tamminga et al., 1979). When intakes were greater, a greater flow of N to the small intestine was a portion of the N ingested when compared to the lower level of intake (Tamminga et al., 1979). This was explained by a lower degradation of N at a higher level of intake due to an increased rate of passage.

Limit-feeding may have some potential downfalls. Hoffman et al., (2007) conducted a study evaluating the effects of limit-feeding on growth, feed efficiency, behavior, and lactation performance. Fifty-four Holstein heifers were assigned to 3 dietary treatments: a control diet based upon NRC (2001) requirements and fed ad-libitum, a limit-fed diet at 90% of the DMI of the control diet, and a limit-fed diet fed at 80% of the DMI of the control diet. The heifers were pen fed with 0.75 m of bunk space per heifer. Heifers fed the control diet spent more time eating than those fed the 90 and 80% limit-fed diets, 19.3, 15.7, and 10.3% of time, respectively (Hoffman et al., 2007). Lying time was also increased in the control fed heifers when compared to the 90 and 80% limit-fed heifers, 60.9, 59.8, and 56.7% of time, respectively (Hoffman et al., 2007). The percentage of time spent vocalizing was also decreased in control fed versus 90 and 80% limit-fed heifers, 0.02, 0.04, and 1.10% of time, respectively (Hoffman et al., 2007). It was concluded that limit-feeding has the potential to cause behavioral changes in gravid heifers because feed is consumed quickly (Hoffman et al., 2007). Therefore, limit-feeding may not be advisable in all heifer rearing operations, especially in situations in which bunk space and animal comfort may be compromised (Hoffman et al., 2007).

Feeding a low nutritive feedstuff to limit-fed heifers may improve behavioral concerns. Kitts et al. (2011) examined the behavioral and growth effects on heifers when a low-nutritive feedstuff was provided with a limit-fed high-concentrate ration. The study utilized 24 heifers that were housed in groups of 4 and pen fed with 0.68 m of bunk space/heifer. A total mixed ration (**TMR**) was fed at 2.02% of BW and wheat straw was not offered, offered on the side, or mixed within the TMR. The two straw diets were comprised of approximately 30% wheat straw on a dry matter (**DM**) basis. Adding straw to the diets increased feeding time, increased rumination time, decreased inactive standing time, and also maintained ADG (Kitts et al., 2011). Therefore, feeding wheat straw can help heifers to satisfy their natural foraging behavior as long as adequate bunk space for each heifer is provided.

Limit-feeding has shown to be advantageous because it improves feed efficiency, decreases the amount of wasted feed, and decreases nutrient excretion all while maintaining growth performance. However, most research regarding limit-feeding has been conducted using corn and soy based diets (Zanton and Heinrichs, 2009). Very limited research has investigated limit-feeding heifers using alternative dietary ingredients such as DDGS.

Puberty

Factors Affecting Growth and Puberty:

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 an increased ADG 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).

Hormonal Control of Puberty

The endocrine system is functioning and begins the production of hormones crucial to puberty attainment before puberty occurs. Within the endocrine system, estradiol employs negative feedback on gonadotropin releasing hormone and the secretion of luteinizing hormone (**LH**), demonstrating that endocrine hormones are functioning before the onset of puberty (Schillo et al., 1992; Sejrsen and Purup, 1997). Schillo and others (1992) found that an increase in pulsatile LH secretion is the essential event in the onset of puberty and this involves communication among the central nervous system, anterior pituitary gland, and ovary. As the heifer ages, it is thought that the estradiol negative feedback receptors become less sensitive, allowing an increase in LH, and subsequent development of ovarian follicles to the preovulatory stage (Day et al., 1984; Schillo et al., 1992).

Leptin

Leptin has become of interest when considering the onset of puberty. It is a peptide hormone secreted primarily from adipose tissue and serves as an indicator of energy reserve status and therefore may be a link between metabolic status and the onset of puberty (Chilliard et al., 2001; Zieba et al., 2004). The specific effects of leptin on the onset of puberty are not fully understood; however, leptin directly affects gonadotropin releasing hormone (**GnRH**) in mature, fasted cattle (Zieba et al., 2004).

Leptin concentration increases in animals that have increased adipose deposition. Leptin and its effect on reproductive status have been investigated in beef heifers. A linear increase in serum leptin concentration from 16 weeks before puberty until ovulation in beef heifers has been shown (Garcia et al., 2002; Maciel et al., 2004). In dairy heifers, research has demonstrated mixed results. Diaz-Torga et al. (2001) found that there was an increase in the concentration of plasma leptin in prepubertal dairy heifers. However, Block et al. (2003) fed prepubertal dairy heifers one of two TMR containing calcium salts of palmitate or conjugated linoleic acid and found no differences in concentrations of plasma leptin. Another study demonstrated that concentrations of plasma leptin may be affected by total intake and diet energy and protein density (Chelikani et al., 2009). This same study also demonstrated that there does not appear to be an increase in concentrations of plasma leptin at puberty, but instead a threshold of leptin concentrations appears to be important for the attainment of puberty especially in heifers (Chelikani et al., 2009).

Although there has been some research investigating the concentrations of plasma leptin in dairy heifers, there has been very limited research investigating the effect of feeding DDGS in dairy heifer diets on concentrations of plasma leptin. Anderson et al. (2015b) conducted a study with prepubertal dairy heifers fed a corn and soybean based diet, a low-fat DDGS, and a high-fat DDGS and found no differences in plasma leptin concentrations. However, these diets were limit-fed at 2.45% of BW and formulated to be isonitrogenous and isocaloric; therefore, it was theorized that total intake of energy rather that form of energy (starch vs fat) has more influence on leptin concentrations.

Cholesterol

It has been found that increasing the concentration of dietary fat increases the concentrations of plasma cholesterol (Park et al., 1983; Talavera et al., 1985; Thomas et al., 1997). Cholesterol is of interest because it is a precursor to steroid hormones such as estradiol and progesterone that are involved in reproduction. It is known that concentrations of plasma cholesterol undergo cyclic changes through the estrous cycle as demonstrated in miniature swine by Lussier-Cacan et al. (1977). There is a reduction in concentrations of plasma cholesterol during the luteal phase of the cycle indicating an inverse relationship between concentrations of plasma cholesterol and plasma progesterone (Lussier-Cacan et al., 1977). Talavera et al. (1985) further demonstrated this change in cholesterol concentrations over the estrous cycle in cattle and also demonstrated that feeding increased dietary fat changed concentrations of plasma cholesterol and also plasma progesterone. However, increasing dietary fat concentrations requires further research to better understand ovarian function (Talavera et al., 1985). Research in beef heifers has demonstrated that feeding diets high in linoleic acid increased concentrations of plasma cholesterol, but did not affect age or BW at the onset of puberty (Garcia et al., 2003).

Changes in concentration of plasma cholesterol due to protein concentration of the diet have also been investigated. Park et al. (1980) fed dairy heifers a low (12%) or high protein (20%) diet with one of two protein supplements soybean meal or sunflower meal. As dietary protein concentration increased, concentrations of plasma cholesterol decreased suggesting that dietary protein has negative effects on plasma cholesterol. This inverse relationship between dietary protein and plasma cholesterol suggests that cholesterol metabolism may depend upon adequate dietary protein or possibly that there is more cholesterol synthesized during a protein deficiency (Park et al., 1980).

Protein and Reproduction

The effect of dietary protein concentration is well researched in dairy cattle. The luminal environment of the uterus can be affected by elevated blood ammonia and urea from the intake of diets with increased dietary crude protein (**CP**) concentrations (Butler, 1998). Across several studies, excess rumen degradable protein (**RDP**) and rumen undegradable protein (**RUP**) results in decreased fertility. This was a result of the formation of urea altering uterine pH. However, the pH of urea is the same as that of the uterus and so the exact mechanism behind this is not fully understood. When lactating cows were fed a TMR containing 18% CP with balanced RDP and RUP fractions as recommended by NRC (2001), uterine pH was affected by concentrations of plasma urea nitrogen (**PUN**) (Butler, 1998). There was a direct effect on uterine pH when concentrations of PUN ranged from 12 to 24 mg/dL (Butler, 1998). Follicular development and ovulation do not appear to be affected by dietary RDP concentrations; but rather decreased concentrations of plasma progesterone, which is involved in establishing and maintaining pregnancy (Butler, 1998).

Feeding Distillers Grains

Changes in feedstuff prices and feeding strategies of dairy heifers have caused alternative feedstuffs to be considered. The ethanol industry has provided DDGS as an economically favorable alternative. The greater fat and reduced starch concentrations have made it a feedstuff to consider in heifer rations. Because of its energy density, it may be a favorable feedstuff when limit-feeding heifers because the amount consumed could be controlled. However, feeding feedstuffs with greater caloric density may affect the metabolic profile and long-term performance of dairy heifers.

Feeding DDGS to dairy cattle has been well researched (Schingoethe et al., 2009). The additional fat, fermentable fiber, and RUP when compared to corn and soybean meal are thought to be the reason for improvements in feed efficiency (Anderson et al., 2006; Klopfenstein et al., 2008). Distillers grains has slower DM digestibility compared to other feeds which makes it a favorable alternative feedstuff (Abdelqader et al., 2009b; Mjoun et al., 2010b). Abdelqader et al. (2009b) characterized the rate of ruminal degradation of corn germ, DDGS, high protein DDGS, and soybean meal. The ruminal digestibility was greatest for the corn germ, and least for the DDGS. Overall, the DDGS had greater RUP than the corn germ and soybean meal. Mjoun et al. (2010b) conducted a study comparing the ruminal degradation and intestinal digestibility of DDGS and soybean products. The distillers products, especially the dried distillers products were more resistant to ruminal degradation when compared to the soybean products (Mjoun et al., 2010b). This suggests that the fat in DDGS may be degraded more slowly than the fat in other feedstuffs or free oil, which may disrupt fiber digestion in the rumen (Jenkins et al., 1993; Klopfenstein et al., 2008).

Distillers grains can be fed to ruminants in many forms. Distillers wet grains with solubles (DWGS) has demonstrated to maintain or improve growth or lactation performance in ruminants (Abrams et al., 1983; Schingoethe et al., 1999; Anderson et al., 2006; Anderson et al., 2009). A study was conducted to determine the lactation performance of lactating dairy cows fed DDGS or DWGS (Anderson et al., 2006). There were five dietary treatments: a control, DDGS fed at 10% of dietary DM, DDGS fed at 20% of dietary DM, DWGS fed at 10% of dietary DM, and DWGS fed at 20% of dietary DM. Milk production was improved with DWGS when compared to the control diet and maintained when DWGS was compared to DDGS. Cows fed DGWS also had greater milk fat, protein, and MUN than cows fed DDGS.

The increased moisture content of DWGS makes storage more challenging than DDGS. Ensiling DWGS may be a way to store the feedstuff for producers looking to feed it over long periods of time. Anderson et al. (2009) conducted two studies in which DWGS was ensiled alone or with soyhulls. The first study was an ensiling study using laboratory silos, while the second was a feeding study to determine the growth performance of heifers fed ensiled DWGS. The ensiling study had three treatments, 100% DWGS, 85% DWGS and 15% soyhulls, and 70% DWGS and 30% soyhulls. Based on the ensiling study, the 70% DWGS and 30% soyhulls treatment was chosen to be further evaluated in the heifer feeding study. Heifers were fed one of three dietary treatments, a control, a low ensiled DWGS with soyhulls fed at 24.4% of dietary DM, or high DWGS with soyhulls fed at 48.6% of dietary DM. Heifer withers height, hip height, heart girth, and body length were similar among treatments. Average daily gain was also similar, suggesting that heifers were growing at similar rates. Dry matter intake decreased with increasing concentration of DWGS. The combination of similar growth among treatments and decreased DMI with increased concentration of DWGS improved the feed efficiency for the high ensiled DWGS with soyhulls treatment.

Dried distillers grains with solubles have been shown to be a replacement for corn and soybean meal in dairy heifer diets without causing changes in ADG or negative post trial performance (Anderson, 2015a, b, c). Anderson et al. (2015a, b, c) limit-fed a corn, soybean meal control diet, low-fat DDGS with corn, or a higher-fat diet containing traditional DDGS to growing dairy heifers. Growth performance, nutrient utilization, metabolic profile, onset of puberty, and lactation performance were investigated. There were no differences in any of the growth parameters among treatments (Anderson et al., 2015a). However, the replacement of starch from corn with fat from DDGS in the diets of dairy heifers resulted in decreased age and BW at the onset of puberty, despite similar ADG (Anderson, 2015b). Providing energy in the form of fat from DDGS also improved digestion and utilization of neutral detergent fiber (**NDF**) and CP compared to providing energy in the form of starch as in the control diet. This suggests there may be an interaction between fat from DDGS and heifer development.

Compared to dairy heifers, feeding DDGS to beef heifers has been well researched. It has been shown to maintain or improve growth and reproductive parameters. When beef heifers were fed high levels of DDGS, age and BW at puberty were not affected (Martin et al., 2007). Artificial insemination conception and pregnancy rates were also improved in heifers fed DDGS compared to a dried corn gluten feed and whole corn germ based control supplement with equal energy (Martin et al., 2007).

Fatty Acid Profile of Distillers Grains

The most prevalent fatty acid in DDGS is typically linoleic acid (C18:2), comprising approximately fifty percent of the fat (Leonardi et al., 2005; Anderson et al., 2006; Anderson et al., 2009; Anderson et al., 2015b). Linoleic acid can be further elongated to arachidonic acid (C20:4) and cholesterol. Arachidonic acid is a precursor for prostaglandins within the body, while cholesterol is a precursor for steroid hormones such as estradiol and progesterone as previously described. Therefore, feeding DDGS may alter the metabolic profile. There is also some speculation that fatty acid profile may alter mammary development by increasing mammary epithelial cell proliferation.

There is very limited research investigating fatty acid profile and growing dairy heifers. McFadden et al. (1990) fed diets with polyunsaturated fatty acids (**PUFA**) to sheep and found increases in pubertal mammary growth; however, the mechanism behind this action was not fully understood. Thibault et al. (2003) fed dairy heifers linoleic acid, which is found in soybean oil from birth to six months of age and found no differences in growth of mammary development. Anderson et al. (2015d) found that dietary linoleic acid increased in diets containing DDGS. Concentration of plasma linoleic acid was also elevated in heifers fed DDGS compared to a control diet; however, there were no differences in concentrations of plasma arachidonic acid.

Reduced-Fat Distillers Grains

Reduced-fat DDGS has been shown to support milk production in lactating dairy cows (Christen et al., 2010; Mjoun et al., 2010a; Ramirez-Ramirez et al., 2016). Christen et al. (2010) fed lactating dairy cows a high protein, reduced-fat DDGS, soybean meal, canola meal, or traditional DDGS. The reduced-fat DDGS in this study was approximately 3.4% ether extract (**EE**). Feeding reduced-fat DDGS maintained milk production and improved milk fat and protein percentage (Christen et al., 2010). Mjoun et al. (2010a) investigated the effects of feeding increasing amounts of reduced-fat DDGS on the lactation performance of dairy cows. Cows were fed diets with 0, 10, 20, or 30% of dietary DM as reduced-fat DDGS. The reduced-fat DDGS was approximately 3.5% EE. There were no differences in DMI among treatments; however, increasing dietary amount of reduced-fat DDGS tended to increase energy corrected milk (**ECM**) and fat corrected milk. Feed efficiency also increased with increasing amounts of reduced-fat DDGS. There was a linear effect for plasma cholesterol with cows fed increased amounts of reduced-fat DDGS having greater concentrations of plasma cholesterol. When reduced-fat DDGS was compared to traditional DDGS in lactating dairy cow diets there were no differences in DMI or milk production; however, fat % and total milk fatty acids increased with reduced-fat DDGS. Total-tract digestibility of DM, organic matter (**OM**), and CP were also improved with reduced-fat DDGS compared to DDGS.

Very limited research has been conducted feeding DDGS to dairy heifers in which some of the fat has been removed. Schroer et al. (2014) fed heifers that were approximately 5 months of age one of three diets: a control, DDGS, or reduced-fat DDGS diet. Heifers were fed for 12 weeks and intake, feed efficiency, and growth were measured. However, this study incorporated reduced-fat DDGS at only 20% of the diet DM. Heifers fed the reduced-fat DDGS had similar ADG, feed efficiency, hip height, and withers height as heifers fed the control diet and DDGS. This demonstrated that reduced-fat DDGS did not negatively affect heifer growth and that reduced-fat DDGS is a viable feed source for dairy heifers (Schroer et al., 2014).

Anderson et al. (2015a, b, c) also limit-fed heifers a control, low-fat DDGS, or high-fat DDGS and found that ADG and body frame measurements did not differ between treatments. Further investigation of the metabolic profile and onset of puberty of these heifers demonstrated that energy status was maintained and onset of puberty was not different. Feeding DDGS also maintained or improved lactation performance in these heifers.

Feeding DDGS to dairy heifers has been limited to high forage diets (Anderson et al., 2009; Anderson, 2015a, b). 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 prepubertal dairy heifer rations. In other words, research has not been conducted in which DDGS has been the main concentrate in limit-fed dairy heifer rations. The high fat content of traditional DDGS, which is typically 10-15% EE, made this feeding strategy difficult. However, the development and availability of DDGS that has some of the fat removed through centrifugation should allow it to be incorporated into the diet at much greater proportions.

Feeding DDGS in which some of the fat has been removed may result in changes in the metabolic profile and metabolism of growing dairy heifers. The fatty acid profile of the DDGS, especially the increased concentration of linoleic acid, may result in changes that could be reflected in the onset of puberty. Increases in concentrations of plasma cholesterol may also be demonstrated as dietary fat concentrations are increased. This could also play a role in the synthesis of reproductive hormones and the onset of puberty. Changes in the onset of puberty could result in heifers reaching puberty sooner, becoming eligible to be bred at a younger age, and entering the lactating herd where there is a quicker return on investments. However, the interaction between diet and metabolic profile warrants further research.

Conclusion

Dairy heifer nutrition strategies can differ according to producer goals. However, heifer nutrition and growth can have long term effects on lifetime productivity and milk production. In order to maximize lactation performance, heifer ADG should be limited to 0.8 kg/d (Zanton and Heinrichs, 2005) and heifers should calve at 23-24 months of age (Ettema and Santos, 2004).

In order to meet the ADG and calving goals for heifers, there are many nutrition and management decisions that must be made that may further affect the metabolic profile, mammary development, and onset of puberty in the heifers. Diet may affect metabolic profile which may play a role in the onset of puberty of the heifers (Funston et al., 2012). Diet and ADG may also affect mammary development that will influence future milk production (Van Amburgh et al., 1998; Zanton and Heinrichs, 2005). Therefore, there are many variables to consider when raising heifers.

Due to the expanding ethanol industry, DDGS has been made available as an alternative feedstuff that has been demonstrated to maintain or improve production performance in dairy cattle. In the Midwest, the low cost and availability of DDGS make it a viable alternative protein and energy source. The higher fat content of DDGS compared to traditional feed ingredients, such as corn and soybean meal, made it difficult to incorporate into dairy heifer diets at high inclusion rates. The recent development of DDGS, in which some of the fat has been removed through centrifugation, should allow for its incorporation into the diet at much greater proportions. The replacement of forage with DDGS could be valuable because it has the potential for producers to increase utilization of this ethanol co-product in dairy heifer rations and may have the benefits shown by others with limit-feeding.

The long term goal of this research is to determine the optimal inclusion amount of the replacement of energy and protein from forage with the energy and protein from DDGS. This will be evaluated by determining the effects on growth performance when DDGS was fed as the major concentrate ingredient at greater proportions of the diet than previously researched. Secondary objectives are to determine the effects of diets on rumen fermentation and total tract digestibility. Additional objectives are to determine the effect of increasing the inclusion amount of DDGS in replacement of forage on the metabolic profile. This is important because changes in metabolic profile may reflect changes in onset of puberty, as well as first lactation performance. The next objective was to determine the effect of feeding DDGS with ad libitum grass hay on heifer growth, rumen fermentation, and total tract digestibility of nutrients. Since DDGS has improved animal efficiency in many studies, it may prove to be a favorable alternative feedstuff for producers to increase efficiency when compared to corn and soybean meal in heifer diets. It was hypothesized that limit-feeding diets with DDGS as the primary concentrate ingredient would maintain growth performance, improve gain: feed and nutrient digestibility, as well as shift, but maintain the overall metabolic profile of growing dairy heifers without having a detrimental effect on the attainment of puberty or lactation performance.

CHAPTER 2:

FEEDING 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

Abstract

The objective of this study was to determine the effects of increasing dietary concentration of distillers dried grains (**DDGS**) in dairy heifer rations. A 16-wk randomized complete block design study was conducted using 48 Holstein heifers (199 ± 2 d of age; BW 206 ± 2 kg) to evaluate effects of dietary treatment on dry matter intake (**DMI**), average daily gain (**ADG**), growth performance, rumen fermentation, and nutrient digestibility. Treatments were 1) 30% DDGS with the diet fed at 2.65% of body weight (**BW**) (**30DG**), 2) 40% DDGS with the diet fed at 2.50 % of BW (**40DG**), and 3) 50% DDGS with the diet fed at 2.35% of BW (**50DG**). The remainder of the diet consisted of grass hay and 1.5% mineral mix. Heifers were individually limit-fed using Calan gates. Heifers were weighed every 2 wk and ration amount offered was adjusted accordingly. Frame measurements and body condition score (**BCS**) were recorded every 2 wk. Rumen fluid was collected via esophageal tubing during wk 12 and 16 for pH, ammonia-N, and volatile fatty acids (**VFA**) analysis. Total tract digestibility of nutrients was evaluated during wk 16 using fecal grab sampling. There were no treatment by week interactions for any of the growth parameters measured and growth parameters did not differ among treatments. Heifer DMI linearly decreased with increasing concentrations of

DDGS. Body weight and ADG were similar among treatments, while gain: feed linearly increased across treatments, with a tendency for treatment by time interaction. As the dietary concentrations of DDGS increased, rumen ammonia-N linearly increased. Acetate proportion and acetate: propionate linearly decreased as DDGS increased, while propionate linearly increased. There were treatment by time interactions for propionate proportion and acetate: propionate. Increasing dietary concentrations of DDGS linearly increased total tract digestibility of DM, organic matter (**OM**), and crude protein (**CP**). Limit-feeding diets with greater concentrations of DDGS improved gain: feed and total tract digestibility of DM and CP, and maintained frame growth without increasing BCS. Results demonstrated that replacing forage with DDGS at up to 50% inclusion rate in limit-fed rations can maintain heifer growth performance.

Keywords: distillers grains, dairy heifer, growth performance

Introduction

Previous research has demonstrated that feeding dried distillers grains with solubles (DDGS) improves feed efficiency in ruminants (Anderson et al., 2006; Klopfenstein et al., 2008; Schingoethe et al., 2009). 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 factors of the improvement in animal production (Schingoethe et al., 2009). Feeding DDGS has been well researched in beef heifers (Klopfenstein et al., 2008); however, there is relatively little research which focuses on feeding distillers grains to growing dairy heifers. Previous research has found that distillers wet grains with solubles ensiled with soyhulls or corn stalks when fed in ad libitum rations to dairy heifers increased average daily gain (ADG) compared to control diets (Anderson et al., 2009; 2015d). Diets with full-fat DDGS or low-fat DDGS, included at approximately 20 or 30 % of DM, have also been demonstrated to maintain ADG and overall growth performance in dairy heifers compared to control diets containing corn and soybean meal when fed ad libitum (Schroer et al., 2014) or limit-fed (Anderson et al., 2015a) .

Diets typically used for limit-feeding are proportionately high in concentrates and are more nutrient dense, allowing an increase in energy and nutrient utilization efficiency while decreasing nutrient loss in fecal matter (Zanton and Heinrichs, 2007). However, we are not aware of any research that examined the effect of DDGS as the primary concentrate ingredient in limit-fed dairy heifer diets. Therefore, the main objective of this study was to determine the effects on growth performance when DDGS was fed as the major concentrate ingredient at greater proportions of the diet than previously researched.

Secondary objectives were to determine the effects of diets on rumen fermentation and total tract digestibility. Additionally, limit-feeding was implemented to avoid excessive ADG that could be caused by increased dietary proportion of DDGS. We hypothesized that increasing the dietary concentration of DDGS would maintain heifer growth performance due to limit-feeding and there would be changes in rumen fermentation as DDGS replaced forage in the diets. We also hypothesized that gain: feed and nutrient utilization would increase with increasing concentrations of DDGS.

Materials and Methods

All procedures and animal use were 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 d of age; 206 ± 2 kg) 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 within blocks. Heifers were added to the study based on farm calving rates and were introduced in multiples of six with a target age of 7 months. Heifers were acclimated to the barns and feeding system for approximately two wk followed by an experimental feeding period of 16 wk.

Treatment diets (Table 1) were: 1) 30% DDGS with the diet fed at 2.65% of body weight (**BW**) (**30DG**), 2) 40% DDGS with the diet fed at 2.50 % of BW (**40DG**), and 3) 50% DDGS with the diet fed at 2.35% of BW (**50DG**) on a DM basis. The remainder of the diets consisted of grass hay and 1.5% mineral mix. Diets were formulated using the NRC (2001) to meet a target ADG of 0.8 kg/d when fed to a 250 kg BW Holstein heifer and to provide similar energy intakes. The 250 kg BW was a pre-estimated average BW for heifers during the study based on age and herd data. On the last two d of each two wk interval, heifers were weighed and then amount of feed offered was determined for the next two wk. Amount of each ration offered was also adjusted using DM analysis of feedstuffs.

In order to avoid variation in production within plant and over time, DDGS was purchased in two batches, one at the beginning of the experiment, and a 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 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 to accommodate available animals and pen space. Heifers were observed daily for health problems and treated according to routine management practices at the DRTF.

Heifers were housed in pens of 6 heifers each. Each pen had an inside roofed area (7 m × 4 m) and an outside dirt exercise lot (7 m × 23.5 m). The inside areas of the pens were a bedded pack, and were bedded with straw once every 2 wk. Because the consumption of bedding material can be a concern when limit-feeding, pens were only bedded once every 2 wk. Each pen was provided with water ad libitum. Heifers were fed once daily at 0830 h using the Calan gate feeding system (American Calan Inc., Northwood, NH) and individual intakes were measured. Bales of hay were coarsely pre-ground with a vertical tub grinder to ease hand mixing. Diet components were individually weighed and hand mixed for each heifer. The mineral mix was mixed with the DDGS before mixing with the grass hay. Because heifers were limit-fed and were expected to consume all feed, particle sorting was a minor concern. Any orts were weighed and recorded every morning before feeding. Samples of DDGS and grass hay were taken each wk 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 measured on 2 consecutive d approximately 4 h post-feeding at the beginning of the study and then every 2 wk 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 wk 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.

Rumen fluid was sampled from each heifer on 2 consecutive days during wk 12 and 16 approximately 4 h 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 pH meter (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, IL) and 2 aliquots (10 mL) 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.

For analysis of total tract digestibility, fecal samples were collected during wk 16 of the feeding period. Acid detergent insoluble ash (**ADIA**) was used as an internal digestibility marker. Orts and fecal grab samples were collected during 3 consecutive d in wk 16 and stored at - 20°C until processing and analysis. Fecal sampling time points were scheduled so that the samples represented every 3 h in a 24 h feeding cycle.

Laboratory Analysis

Total dietary nutrient concentrations were calculated based on analysis of grass hay and DDGS for each treatment. Feed samples were dried for 24 h at 105°C for DM analysis in order to adjust dietary ingredient inclusion rates and determine DMI. Samples of DDGS and grass hay were collected once weekly and frozen at -20°C until analysis. Samples of DDGS and grass hay were thawed and samples from 4 consecutive wk were composited on an as-fed basis by weight. Composite samples were dried in duplicate for 48h 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. Philadelphis, 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 h at 450°C in a muffle furnace (AOAC 17th ed., method 942.05; 2002). 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). Analysis was conducted using both solvents because differences in polarity between the solvents resulted in different extraction values. Diethyl ether is the recommended solvent for most animal feeds. Because diethyl ether tends to overestimate EE in DDGS, petroleum ether is the recommended solvent for EE analysis (Thiex, 2009). 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 DDGS were further composited into four or five month composites and sent to a commercial laboratory (Dairyland Laboratories, Inc. Arcadia, WI) for analysis of minerals (Ca, Cl, Mg, P, K, Na, S, Fe, Mn, Mo, and Zn) and starch. 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).

Rumen fluid samples preserved with sulfuric acid were thawed and centrifuged at 30,000 × g for 20 minutes at 4°C (Centrifuge: Eppendorf 5403, Eppendorf North America, Hauppauge, NY) and analyzed for ammonia N using a colorimetric assay performed 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 at 4°C and analyzed for acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations 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 100:1 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. Available orts were collected each 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 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 (2002). Digestibility calculations were determined according to Merchen (1988).

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The MEANS procedure of SAS was used to estimate the means and standard errors of the nutrients of the monthly feed composites.

Heifer intake, growth data, and rumen fermentation parameters were analyzed as a randomized complete block design with week as the repeated measure and the term heifer (block) as the subject using the PROC MIXED procedures of SAS (Littell et al., 2006). The model included treatment, wk, and treatment × wk interactions. Initial body size measurements and BW were included as covariates within the model. Akaike's criterion was used to determine the most suitable covariance structure in repeated measures for each parameter. Covariance structures tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. Compound symmetry resulted in the least absolute Akaike's values and was used for the final model. 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 determine average change per day for ADG and body frame measurements. The *P values* for the interaction of treatment and time using MIXED analysis were used to determine significance of change per day among treatments (Kutner et al., 2004). Gain to feed ratio was calculated as the ratio of ADG (slope of BW regression) to DMI for each treatment. For comparison of analyses, ADG and gain: feed were also calculated based on 2 wk interval data and analyzed using MIXED procedures with repeated measures similar to frame size parameters.

The MIXED procedures of SAS were used for the analysis of total tract digestibility of nutrients. The model included treatment with block included as a random variable because samples were analyzed from a single time period.

Results and Discussion

Feed Analysis

The nutrient composition of the individual ingredients used in the experimental diets is presented in Table 2. Because the DDGS was purchased in two large batches, nutrient composition of the DDGS did not vary much over the duration of the study; however, there was some variation in the nutrient composition of the grass hay during the experiment.

Average nutrient composition of the experimental diets is presented in Table 3. The nutrient composition was based on individual ingredient analysis during the course of the study. The dietary CP concentration increased with increasing concentrations of DDGS as expected due to experimental design. The EE concentrations of the diets increased with increasing concentrations of DDGS. Concentration of NDF decreased with increasing concentrations of DDGS. Experimental diets had greater NDF than formulated due to changes in grass hay quality during the study. Starch concentration increased with increasing dietary concentration of DDGS; however, starch concentrations were very low across all diets. Therefore, the other nutrients including fat, fiber, and protein rather than starch were the major energy sources in the diets and we speculate that some protein was used as gluconeogenic precursors (Fahey and Berger, 1988). As the concentration of DDGS in the diet increased, energy density of rations increased, justifying the use of limit-feeding to avoid overconsumption and high ADG as seen by Anderson et al. (2009 and 2015d).

Differences in nutrient composition of the diets were further reflected in nutrient intakes (Table 4). Crude protein, EE, starch, and sulfur intake increased with increasing concentration of DDGS; however, NDF intake decreased. Sulfur intake increased across treatments; however, sulfur toxicity, which can occur when feeding large amounts of DDGS (Schingoethe et al., 2009), was not an issue in this study. Sodium bicarbonate and limestone were included in the experimental diets for buffering and to mitigate any risk of sulfur toxicity. Additionally, water supplied to heifers was from a municipal water treatment plant and had low sulfate concentration (approximately140 mg/L). Despite differences in nutrient composition and intake among treatments, ME and NE_g intakes were similar among treatments which is consistent with similar ADG (Table 5). Actual intakes as a percentage of BW (Table 5) were less than the prescribed feeding rate. Despite being limit-fed, heifers did have some orts, especially in the few days directly following ration increases. Additionally, heifers were weighed and then amount of feed offered was determined for the next two wk, so as heifers were always gaining weight daily the DMI as percentage of BW was constantly decreasing during the 14 d intervals.

Heifer Performance

Body weight, DMI, and gain: feed results are presented in Table 5. The BW and ADG found via regression and based on two week interval calculations were similar among treatments. The ADG in this experiment was greater than the target recommendation of 0.8 kg/d (Zanton and Heinrichs, 2005). Because this research was intended to build upon the research conducted by Anderson et al. (2015a; b), the NRC

(2001) model was used to formulate the diets. The results from this experiment and Anderson et al. (2015a, b) suggest that the NRC (2001) model overestimates the energy requirements of growing dairy heifers or underestimates energy provided by DDGS. The current experiment and Anderson et al. (2015a; b) demonstrate that heifers can be limit-fed diets with DDGS to control ADG, but amounts offered should be less than NRC (2001) recommendations.

Dry matter intake decreased and gain: feed from regression analysis and calculated based on 2 wk intervals (Table 5) increased across treatments. Nutrient density of the diet increased with increased DDGS, therefore less feed was required to achieve similar ADG. This difference in DMI and gain: feed is consistent with previous experiments that controlled the nutrient intake in diets differing in forage concentration (Hoffman et al., 2007; Lascano and Heinrichs, 2009; Zanton and Heinrichs, 2009). As originally hypothesized, 50DG had the greatest gain: feed and it linearly decreased with greater proportion of forage.

Frame size measurements and BCS are presented in Table 6. Based on genomic data, 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, $P = 0.57$); therefore it was not used as a covariate term for growth performance. There were no treatment by week effects for any of the frame growth parameters measured. Frame size measurements increased over time, but there were no differences among treatments. There were also no differences in change per day for any of the frame growth measurements, suggesting that all treatment diets provided adequate ME and protein to maintain growth during the experimental period. There were no differences among treatment for BCS (Table 6). Throughout the experiment, heifers maintained BCS close to 3.0 with a tendency ($P = 0.09$) for BCS to decrease over time, indicating that heifers were not accumulating excess adipose tissue. These results are consistent with findings by Anderson et al. (2015a) who fed low-fat and traditional DDGS compared to a control diet.

Rumen Fermentation

Rumen fermentation characteristics are presented in Table 7. There was a treatment by week interaction for isobutyrate concentration, propionate molar percentage, and acetate to propionate ratio, and tendencies for a treatment by week interaction was observed for acetate, valerate, and total VFA concentrations. Propionate concentration linearly increased as the dietary concentration of DDGS increased, while butyrate concentration and acetate to propionate ratio linearly decreased with increasing dietary concentration of DDGS. The propionate molar percentage also increased, while acetate and butyrate molar percentages decreased. The shift in molar VFA concentrations is a result of differences in dietary forage concentrations, suggesting a shift in bacterial species population in the rumen. Acetate production within the rumen is the result of 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 DDGS increased is consistent with other studies that fed heifers diets differing in concentrate proportions (Lascano et al., 2009; Suarez-Mena et al., 2015). This also suggests that heifers fed greater concentrate to forage ratios of DDGS may have more efficient rumen fermentation as shown by a greater proportion of propionate production.

Production of propionate results in less carbon loss as methane or carbon dioxide compared to acetate (Fahey and Berger, 1988). This theory is also supported by increased gain: feed as DDGS was increased in the diet.

Rumen ammonia-N concentration linearly increased as the dietary concentration of DDGS increased. Suarez-Mena et al. (2015) fed increasing concentrations of DDGS in replacement of canola meal at two forage concentrations and found that ammonia-N tended to be greater for high forage diets as the result of lower microbial activity for microbial protein synthesis. However, diets in that experiment had greater starch and NFC concentrations and lower CP than diets in the current experiment. Ammonia is used for protein synthesis within the rumen and accumulates when protein degradation exceeds microbial requirements (NRC, 2001). The supply of fermentable carbohydrates also can affect the assimilation of N by rumen bacteria (Nocek and Russell, 1988; Bach et al., 2005). Therefore, low dietary starch concentrations and the increased CP concentrations may explain the high rumen ammonia-N among treatments. Research using lactating dairy cows has also shown that increasing the dietary CP results in increased concentrations of ruminal ammonia-N (Hristov et al., 2004). Additionally, rumen fluid samples were taken at a single time point, approximately 4 h post-feeding, when ammonia-N concentrations are potentially at their greatest (Owens and Zinn, 1988). Further research with more frequent sample collections may be warranted to determine if rumen ammonia-N concentrations fluctuate or if they remain high throughout the day when dairy heifers are limit-fed diets high in DDGS.

Total Tract Nutrient Digestion

Total tract nutrient digestibility is presented in Table 8. Digestibility of NDF and ADF was similar among treatments, whereas digestibility of DM, OM, and CP linearly increased with increasing concentrations of DDGS ($P < 0.01$). This is because grass hay has less TDN compared to DDGS (NRC, 2001). With greater DDGS, the greater amounts of fat consumed potentially could interfere with fermentation because of the effects of unsaturated lipids on microbial growth and negatively affected the digestibility of nonlipid energy sources (Jenkins, 1993; NRC, 2001). However, even with 50% inclusion rate of DDGS in the diet, total diet EE concentration was less than 8%, which is thought to be the upper limit before fat concentration begins to have negative effects on the rumen degradation of fiber and DM (Palmquist, 1994; NRC 2001). Anderson et al. (2015a) speculated that the fat from DDGS is bound within the feed particle and had less severe effects on digestion of nutrients because it is slowly introduced in the rumen.

The total tract digestibility of CP in the current study is consistent with previous research (Anderson et al., 2015a), who found that when dairy heifers were fed full-fat DDGS at 33% of diet DM the total tract digestibility of CP was 73%. In the current experiment, the total tract digestibility of CP was 86% for the 50DG treatment, which is greater than that reported by Anderson et al. (2015a); however, the 30DG treatment was similar.

Conclusion

In agreement with our hypothesis, limit-feeding diets containing DDGS up to 50% of DM maintained growth performance of dairy heifers based on BW, ADG, and frame growth. There were no differences in BW and ADG was maintained among treatments. However, ADG was greater than NRC (2001) predictions for all treatments, but heifers did not accumulate excess adipose tissue as demonstrated by a tendency to decrease BCS during the course of the study. In addition, increasing the dietary concentration of DDGS in replacement of forage increased gain: feed and nutrient digestibility of DM, OM, and CP. Overall, this research indicated that DDGS can be fed as part of limit-fed rations for growing dairy heifers at up to 50% of dietary DM and result in increased feed efficiency and maintained growth performance compared to inclusion at 30 or 40% of diet DM.

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Table 1. Ingredient composition of treatment diets with increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay limit-fed to growing replacement Holstein dairy heifers

	Treatment[1]		
Ingredient[2], % DM	30DG	40DG	50DG
DDGS	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 DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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 distillers dried grains with solubles (**DDGS**) used in the treatment diets limit-fed to growing Holstein dairy heifers

	Grass hay		DDGS	
Item[1]	Mean	SE	Mean	SE
DM[2], %	86.3	0.31	86.9	0.35
Ash[2]	8.76	0.328	4.68	0.037
OM[2]	91.2	0.33	95.3	0.03
CP[2]	9.81	0.417	33.6	0.18
ADF[2]	37.8	0.50	10.0	0.35
NDF[2]	66.4	0.62	29.8	0.38
EE (Diethyl)[2]	1.87	0.101	12.9	0.13
EE (Petroleum)[2]	1.05	0.102	7.80	0.079
NFC[2,3]	14.0	0.90	24.1	0.33
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 4- or 5-month composites (n = 3).

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Table 3. Nutrient composition of treatment diets with increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay limit-fed to growing Holstein dairy 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 DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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 4- or 5-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 limit-fed increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) 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	0.169	0.03	<0.01	0.97	<0.01	0.84
OM[3]	5.91	5.68	5.37	0.155	0.06	<0.01	0.98	0.02	0.83
CP[3]	1.09	1.19	1.26	0.033	<0.01	<0.01	0.04	<0.01	0.69
NDF[3]	3.53	3.15	2.75	0.085	<0.01	<0.01	<0.01	<0.01	0.93
ForageNDF[3]	2.95	2.41	1.88	0.065	<0.01	<0.01	<0.01	<0.01	0.95
NonforageNDF[3]	0.58	0.74	0.87	0.021	<0.01	<0.01	<0.01	<0.01	0.55
EE (Diethyl)[3]	0.33	0.39	0.43	0.011	<0.01	<0.01	<0.01	<0.01	0.64
EE (Petroleum)[3]	0.20	0.23	0.26	0.007	<0.01	<0.01	<0.01	<0.01	0.61
Starch[4]	0.15	0.18	0.20	0.005	<0.01	<0.01	<0.01	<0.01	0.68
Sulfur[4]	0.021	0.024	0.026	0.0007	<0.01	<0.01	<0.01	<0.01	0.66
ME, Mcal/d	14.7	14.8	14.7	0.41	0.96	<0.01	0.99	0.91	0.78
NE$_{G,}$ Mcal/d	5.25	5.59	5.78	0.154	0.06	<0.01	0.37	0.02	0.72

[1] 30% dietary inclusion rate of DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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 4- or 5-month composites (n = 3).

Table 5. Dry matter intake, body weights, and gain to feed ratios for Holstein heifers limit-fed increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay

	Treatment[1]				*P-value*[2]				
Item	30DG	40DG	50DG	SEM	Trt	wk	Trt × wk	L	Q
Age, initial	198.1	200.3	199.2	1.93	0.49				
BW, kg									
Mean	264.0	266.2	266.4	7.15	0.97	<0.01	0.72	0.82	0.91
Initial	206.6	205.1	206.1	1.95	0.85				
Final	307.6	312.5	313.0	7.35					
ADG[3], kg/d	0.89 ±0.071	0.94 ± 0.083	0.97 ± 0.083		0.44				
ADG[4], kg/d	0.91	0.96	0.95	0.043	0.67	<0.01	0.99	0.47	0.60
DMI, kg									
Mean	6.49	6.21	5.84	0.169	0.03	<0.01	0.97	<0.01	0.84
Final	7.75	7.37	7.05	0.178					
Gain:Feed[3]									
Mean	0.141	0.156	0.172	0.0051	<0.01	<0.01	0.09	<0.01	0.93
Gain:Feed[4]									
Mean	0.144	0.159	0.165	0.0063	0.06	<0.01	0.99	0.02	0.56
DMI, % BW									
Mean	2.45	2.33	2.19	0.012	<0.01	<0.01	0.79	<0.01	0.59
Final	2.52	2.36	2.26	0.025					

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

[4] Calculated based on change per two week intervals.

Table 6. Frame size measurements for Holstein heifers limit-fed treatment diets with increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) 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	0.39	0.41	<0.01	0.88	0.28	0.44
Initial	113.5	113.1	114.5	0.32	<0.01				
Final	125.7	127.1	127.1	0.49					
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	0.52	0.97	<0.01	0.93	0.99	0.80
Initial	115.3	116.2	117.3	0.51	<0.01				
Final	130.0	130.1	130.2	0.57					
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	0.47	0.85	<0.01	0.81	0.94	0.57
Initial	130.9	131.2	130.7	0.79	0.76				
Final	149.1	148.9	149.6	0.60					
Change[3], cm/d	0.171 ±0.014	0.170 ±0.018	0.181 ±0.015	-	0.65				
Paunch girth, cm									
Mean	172.6	173.8	172.4	1.33	0.73	<0.01	0.97	0.90	0.44
Initial	163.7	162.0	162.1	1.02	0.16				
Final	180.0	182.8	180.9	1.65					
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	0.80	0.84	<0.01	0.96	0.58	0.95
Initial	101.0	101.6	101.5	0.44	0.30				
Final	118.0	119.0	118.7	0.93					
Change[3], cm/d	0.116 ±0.009	0.123 ±0.011	0.123 ±0.010	-	0.63				
Hip width, cm									
Mean	35.63	35.82	35.76	0.452	0.95	<0.01	0.79	0.83	0.82
Initial	32.19	32.11	32.43	0.153	0.30				
Final	38.18	38.50	38.42	0.476					
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	0.028	0.34	0.09	0.14	0.24	0.37
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 DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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 7. Rumen fermentation parameters of Holstein heifers limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay

	Treatments[1]				*P-values*[2]				
Item	30DG	40DG	50DG	SEM	Trt	wk	Trt × wk	L	Q
pH	6.67	6.54	6.52	0.087	0.42	0.10	0.46	0.22	0.60
NH_3-N, mg/dL	15.4	17.1	19.3	1.03	0.03	0.52	0.25	<0.01	0.84
Acetate, m*M*	43.4	41.9	41.7	1.38	0.63	0.29	0.07	0.38	0.70
Propionate, m*M*	18.1	19.9	22.6	1.03	0.01	<0.01	0.15	<0.01	0.73
Isobutyrate, m*M*	0.87	0.95	0.95	0.037	0.23	0.37	0.03	0.15	0.37
Butyrate, m*M*	8.88	8.58	7.26	0.420	0.02	0.22	0.22	<0.01	0.32
Isovalerate, m*M*	0.48	0.58	0.50	0.029	0.06	0.20	0.53	0.72	0.02
Valerate, m*M*	1.33	1.30	1.24	0.054	0.53	0.07	0.05	0.27	0.82
Total VFA, m*M*	73.1	73.2	74.2	2.43	0.93	0.09	0.08	0.73	0.88
Acetate, m*M*/100m*M*	59.4	57.3	56.2	0.55	<0.01	0.03	0.27	<0.01	0.52
Propionate, m*M*/100m*M*	24.7	26.9	30.4	0.81	<0.01	0.05	0.03	<0.01	0.54
Isobutyrate, m*M*/100m*M*	1.20	1.31	1.28	0.042	0.19	0.48	0.21	0.18	0.21
Butyrate, m*M*/100m*M*	12.2	11.9	9.8	0.50	<0.01	0.64	0.31	<0.01	0.19
Isovalerate, m*M*/100m*M*	0.67	0.80	0.68	0.038	0.05	0.03	0.38	0.80	0.02
Valerate, m*M*/100m*M*	1.80	1.78	1.67	0.058	0.24	0.14	0.22	0.12	0.50
Acetate:Propionate	2.44	2.18	1.90	0.075	<0.01	<0.01	0.02	<0.01	0.86

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

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

Table 8. Total tract digestibility of nutrients for Holstein heifers limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) 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 DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**50DG**).

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

CHAPTER 3:

FEEDING DISTILLERS DRIED GRAINS IN REPLACEMENT OF FORAGE IN LIMIT-FED DAIRY HEIFER RATIONS: EFFECTS ON METABOLIC PROFILE AND ONSET OF PUBERTY

Abstract

The objective of this study was to determine the effect of increasing the inclusion rate of distillers dried grains (**DDGS**) in replacement of forage in limit-fed diets on the metabolic profile and onset of puberty in dairy heifers. A 16-wk randomized complete block design study was conducted using 48 Holstein heifers (199 ± 2 d of age) with three treatments. Treatments were 1) 30% DDGS (**30DG**), 2) 40% DDGS (**40DG**), and 3) 50% DDGS (**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. Jugular blood samples were collected during wk 0, 4, 8, 12, and 16 for metabolite and metabolic hormone analysis. Additional samples were taken during wk 16 for plasma fatty acid analysis. When heifers weighed 200 kg, coccygeal vein blood samples were taken twice per wk for progesterone analysis to estimate onset of puberty. Blood samples continued until cycling was confirmed via ultrasound for the presence of a corpus luteum (**CL**). There was a quadratic response and a linear tendency in the proportion of total fatty acids as linoleic acid. There was also a linear response for plasma concentration of linoleic acid. There was also a linear and quadratic response for arachidonic acid. Overall results for fatty acid analysis demonstrated that total fatty acid and polyunsaturated fatty acids (**PUFA**) concentration in the blood were linearly increased with a quadratic response with 30DG and 50DG having the greatest concentrations. There were no interactions of treatment by wk for any of the metabolites and metabolic hormones measured. Glucose, insulin, insulin-like growth factor-1(**IGF-1**), leptin, and triglycerides were similar across treatments. There was a quadratic response of plasma urea nitrogen and a quadratic response tendency for cholesterol concentration. Age and BW at puberty were similar across treatments. Limit-feeding heifers with greater inclusion rates of DDGS maintained energy status without the accumulation of excess adipose tissue as indicated by leptin. Treatments had no detrimental effects on age or BW at puberty.

Keywords: distillers grains, metabolic profile, dairy heifer

Introduction

Feeding dried distillers grains with solubles (**DDGS**) has been reported to maintain growth performance and improve feed efficiency in cattle compared to feeding corn and soybean meal (Anderson et al., 2006; Klopfenstein et al., 2008; Schroer et al., 2014). Traditional DDGS has been limited to high forage diets due to the high fat content (10-15% ether extract). However, in recent years the fat content of DDGS has been reduced. Most ethanol manufacturers are removing some of the fat through centrifugation. The lesser fat content in DDGS could allow it to be incorporated into the diets of cattle at much greater proportions. Increasing the inclusion rate of DDGS in the diet would alter the nutrient profile of the diet which may cause differences in plasma metabolites and metabolic hormone concentrations. This is of interest because changes in the metabolic profile may cause changes in the onset of puberty in cattle (Perry, 2011; Funston et al., 2012) as well as mammary gland development and future milk production (Van Amburgh et al., 1998; Zanton and Heinrichs, 2005).

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 body weight (**BW**) of 250 to 280 kg (Sejrsen and Purup, 1997). In beef heifers, an increase in average daily gain (**ADG**) can influence the age and weight at which heifers attain puberty with heifers having an increased ADG 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 (Zieba et al., 2005). 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).

Leptin has also become of interest when considering the onset of puberty. When animals have increased adipose deposition, leptin concentration can increase. Leptin and its effect on reproductive status have been investigated in beef heifers. A linear increase in serum leptin concentration from 16 weeks before puberty until ovulation in beef heifers was reported (Maciel et al., 2004). There has been very limited research done on leptin concentrations in growing dairy heifers. Anderson et al. (2015b) conducted a study with dairy heifers fed a corn and soybean based diet, a high protein DDGS diet with less fat, and a traditional DDGS that was higher in fat and found no differences in plasma leptin concentrations.

The main objective of this study was to determine the effect of increasing the inclusion rate of distillers dried grains in replacement of forage in limit-fed diets on the metabolic profile and onset of puberty in dairy heifers. Since metabolic profile changes can affect reproductive development, a secondary objective was to determine if changes in the metabolic profile would reflect changes in the onset of puberty. The main hypothesis was that there would be changes in the metabolic profile especially plasma fatty acid concentrations and cholesterol as inclusion rate of DDGS increased; however, heifers would still maintain energy status because of use of a limit-feeding strategy. It was also hypothesized that there would be changes in the onset of puberty as a result of changes in the metabolic profile.

Materials and Methods

Experimental Design

Samples for this experiment were taken during the previously described feeding study from Chapter 2. Refer to this chapter for details on diets, feeding protocols, animal care and heifer growth performance. All animal use was approved by South Dakota State University Institutional Animal Care and Use Committee. Forty-eight Holstein heifers (199 ± 2 d of age) were used in a randomized complete block design with three treatment diets. The feeding period lasted for 16 wk, beginning during the prepubertal period. Treatment diets (Table 9) were: 1) high forage with 30% of diet as DDGS (**30DG**), 2) moderate forage with 40% of diet as DDGS (**40DG**), and 3) low forage with 50% of diet as DDGS (**50DG**) on DM basis. The forage portion of the diets consisted of grass hay. The amount of feed offered was determined as a percentage of BW and decreased with increasing concentrations of DDGS in order to allow for 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). Diets were formulated using the NRC (2001) to provide similar energy intakes when fed to a 250 kg BW Holstein heifer. Heifers were fed individually using a Calan gate feeding system (American Calan Inc., Northwood, NH). Nutrient composition and average intakes are also provide in Table 9. Complete descriptions of ingredients and nutrient analysis can be found in Chapter 2.

Sample Collection and Analysis

For the analysis of cholesterol, glucose, insulin, IGF-1, insulin, leptin, plasma urea nitrogen, and triglycerides, blood samples were taken on two consecutive days during wk 0, 4, 8, 12, and 16 of the feeding study. Blood samples were taken approximately 4 h post-feeding (1230 h) via venipuncture of the jugular vein into vacutainer tube (Becton, Dickinson, and Company, Franklin Lakes, NJ) containing sodium fluoride (NaFl) and potassium oxalate (K 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 h of collection. 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 for metabolites or hormones, plasma or serum from the two consecutive days during each of the blood sampling weeks (wk 0, 4, 8, 12, and 16) were both analyzed and then averaged for statistical analysis.

Metabolites (cholesterol, glucose, plasma urea nitrogen, and triglycerides) were analyzed with commercially available enzymatic or colorimetric assay kits 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.

Samples were sent to the University of Missouri for IGF-1 and leptin analysis. Plasma concentrations of IGF-1 and leptin were determined via double antibody radioimmunoassay (**RIA**) previously validated within Dr. Duane Keisler’s laboratory and inter- and intra-assay CV were < 5% (Lalman et al., 2000 and Delavaud et al., 2000; respectively).

Insulin was analyzed using a commercially available insulin assay (MP Biomedical) according to the manufacturer’s directions. Increasing volumes of bovine serum (25, 50, 75, and 100, μL) produced a displacement curve that was parallel (P = 0.60) to the standard curve (Slope = 1.93 ± 0.22 for standard curve; Slope = 1.77 ± 0.20 for bovine serum). Addition of known amounts of insulin (35 and 155 μIU/mL) to cow serum were accurately recovered (106%). Interassay and intraassay coefficient of variation was 10.10% and 3.85% respectively, and assay sensitivity was 5.5 μIU/mL.

During wk 16, an extra 8 ml blood sample was collected from each heifer and plasma was collected 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. (2009a). Feed samples for fatty acid analysis were collected and four or five month composites of DDGS and grass hay were analyzed for fatty acid profiles via direct butylation techniques (Abdelqader et al., 2009a). All prepared fatty acid samples were analyzed via GC (Hewlett Packard 6890, Palo Alto, CA) as described by Abdelqader et al. (2009a).

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 was confirmed via ultrasonography (Agroscan AL, Echo Control Medical, Angoulême, France). 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. Plasma progesterone concentrations were determined using a validated RIA procedure as described by Engel et al. (2008). Interassay and intraassay coefficient of variation was 13.3% and 2.46% respectively, and assay sensitivity was 0.4 ng/mL. 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 ovulation had occurred and a CL had formed.

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). Feed fatty acid analysis data was compiled for the four or five month feed composite analysis and standard errors were calculated using the MEANS procedure in SAS. Dietary fatty acid values were calculated based on analysis of the grass hay and DDGS for each treatment over the course of the study. Fatty acid intake and plasma and serum metabolites and metabolic hormones were analyzed as a randomized complete block design with repeated measured using the MIXED procedures of SAS 9.4 with wk as the repeated measure using heifer (block) as the subject (Littell et al., 2006). The model included treatment, wk, and treatment × wk interactions. Initial metabolites and metabolic hormones were included as covariates within the model. Akaike's criterion was used to determine the most suitable covariance structure in repeated measures for each parameter.

Covariance structures tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. Compound symmetry resulted in the least absolute Akaike's values and was used for the final model. The MIXED procedures of SAS 9.4 were used for the analysis of plasma fatty acid profile. The model included only treatment with block included as a random variable as it was based on samples taken during only wk 16 of the study. Puberty data was analyzed as binomial data (cycling or not cycling) by certain criteria for age or weight. Puberty data was also analyzed using repeated measures by 10 d and 10 kg intervals of age and BW. Significance differences among treatments for all analyses 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.

Results and Discussion

Dietary Fatty Acids

The fatty acid profiles of the grass hay and DDGS used in the experimental diets are shown in Table 10. Grass hay had greater concentrations of medium and long chain fatty acids (C10:0, C12:0, C12:1, C16:1, C20:0, and C18:3α), while DDGS 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 acids profiles of these feedstuffs were consistent with those found by Leonardi et al. (2005) and Anderson et al. (2015d) who found that long chain fatty acid concentration increased with increasing inclusion rate of DDGS. Fatty acid profiles of the experimental diets are found in Table 11. 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 DDGS increased. Differences in fatty acid profiles of the diets were further reflected in the fatty acid intake (Table 12). Intakes of medium chain fatty acids (C10:0, C12:0, C12:1) linearly decreased with increasing concentrations of DDGS. However, intake of medium and long chain fatty acids (C14:0, C16:0, C18:1 *cis* 11, and C18:2 *cis* 9, *cis* 12) increased with increasing concentrations of DDGS which 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.

Metabolites and Metabolic Hormones

Average plasma fatty acid 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 and a linear tendency for an increase in the proportion of total fatty acids as linoleic acid (C18:2) with increasing dietary inclusion of DDGS. Linoleic acid was also the greatest proportion of fatty acids across all treatments. Plasma concentration of linoleic acid also linearly increased with increasing dietary concentrations of DDGS. 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 dietary concentrations of DDGS as expected by experimental diets. There was also a linear and quadratic effect for arachidonic acid (C20:4) which is the precursor for the synthesis of prostaglandins (Funston, 2004) and may play a role in the onset of puberty. Overall results for fatty acid analysis demonstrated that total fatty acid and PUFA concentration in the blood were linearly increased with a quadratic effect as dietary concentration of DDGS increased. More specifically, there was a marked increase in plasma total fatty acids and PUFA in the heifers fed 50DG, and less of a difference between heifers fed the 30DG or 40DG diets. These results are consistent with Anderson et al. (2015b) who also found that plasma linoleic acid proportion was increased in diets with increased dietary fat concentrations from DDGS. Plasma linoleic acid concentration also increased in diets with increased dietary fat from DDGS (Anderson et al., 2015b). Additionally, there was an increase in palmitic acid in diets containing DDGS in the experiment conducted by Anderson et al. (2015b). However, 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 wk 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 which are comprised of fatty acid chains and glycerol and serve as a major storage form of fat in the body. This is different from the results found by Park et al. (1983) in which heifers were fed diets with increasing levels dietary fat. Increasing the inclusion rate of sunflower seeds (20 to 30% of dietary DM) resulted in elevated triglyceride concentrations in the blood (Park et al., 1983). However, there was a quadratic tendency for plasma cholesterol. Other researchers have reported increases in plasma cholesterol with increased dietary fat concentrations (Park et al., 1983; Talavera et al., 1985; Thomas et al., 1997; Funston, 2004). The increased plasma cholesterol concentration in the 30DG treatment is speculated to be a result of increased rumen acetate concentration and proportion (Chapter 2). Plasma cholesterol concentration drastically increased during the first 4 wk of the trial and then continued to gradually increase over the remainder of the feeding period with the exception of 40DG which decreased during wk 16 (Figure 1). Cholesterol is an important metabolite in reproduction because it is a precursor for steroid hormone synthesis. Progesterone may be more easily affected by plasma cholesterol concentration than other steroid hormones such as estradiol (Talavera et al., 1985). In cattle, the rate-limiting step in progesterone synthesis is the conversion of cholesterol to pregnenolone, and cholesterol as well as pregnenolone are needed for estradiol synthesis (Talavera et al., 1985). However, due to the scope of this study, blood was not sampled at the frequency necessary to monitor progesterone concentrations throughout the estrous cycle.

Concentrations of serum glucose did not differ across treatments. Previous research has reported decreases in blood glucose concentration in dairy heifers fed diets with elevated fat concentrations (9.2 and 13.1%) (Park et al., 1980). This was attributed to the decreased availability of glucogenic nutrients in diets with elevated fat concentrations as well as changes in rumen fermentation resulting in a greater acetate: propionate ratio with decreased propionate production, a gluconeogenic precursor (Park et al., 1980). The lack of differences in the current study may be attributed to the dietary treatments providing enough energy and CP. There was a treatment effect for plasma urea nitrogen with increasing concentrations of DDGS. Over the course of the trial, plasma urea nitrogen increased. This increase can be explained by the increase in dietary crude protein across treatments (Table 9). Other research has reported an inverse relationship between dietary CP and cholesterol concentration (Park et al., 1980). However, diets in the current study were not deficient in CP as reflected by plasma urea nitrogen and dietary CP differences cannot explain differences in plasma cholesterol concentration.

There were no differences in plasma insulin, IGF-1, or leptin concentrations. However, the concentrations of plasma IGF-1 increased across treatments over the course of the study (Figure 2). Plant oils with greater concentrations of PUFA have led to increases in serum concentrations of cholesterol and insulin to enhance IGF-1 production by luteal tissue in vitro (Talavera et al., 1985; Wehrman et al., 1991; Ryan et al., 1995). Since DDGS has significant concentrations of PUFA this may explain the numerical increase in plasma IGF-1 concentration in the 50DG diet; however, results were not significant. Also, IGF-1 is capable of activating insulin receptors at greater concentrations, but no differences were reflected in plasma insulin concentrations. A major component of the fatty acid profile of DDGS is linoleic acid, resulting in the production of propionate in the rumen that can be used in gluconeogenesis (Palmquist, 1981; Chalupa et al., 1986). In the rumen, linoleic acid is converted to glycerol and free fatty acids. Glycerol can then be converted to propionate and become gluconeogenic (Chalupa et al., 1984). However, increased gluconeogenesis did not produce differences in plasma concentrations of insulin in the current study. The results from the current study agree with those reported by Anderson et al. (2015d) where DDGS was limit-fed at up to 30% of dietary DM and no differences in plasma insulin were reported, suggesting that short-term energy status was maintained by feeding DDGS compared to corn and soybean meal.

Long term energy status was maintained as demonstrated by plasma leptin (Zieba et al., 2005). Importantly, throughout the study plasma concentrations of leptin remained fairly constant (Figure 3), indicating no treatment was gaining increased adipose compared to the others. This is in agreement with previous research on differing 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 was supported by the maintenance of growth (Chapter 2) suggests that heifers are using the fat and protein from DDGS as energy in replacement of forage fiber and protein when utilizing a limit-feeding strategy.

Puberty

Mean age and BW at puberty are presented in Table 16. Despite no differences, 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. Holstein heifers attain puberty between 9 and 11 months of age at a BW of 250 to 280 kg (Sejrsen and Purup, 1997). However, others have reported BW close to 300 kg at the onset of puberty (Zanton and Heinrichs, 2007; Chelikani et al., 2009). Heifers in the current study averaged 253.9 kg of BW at the onset of puberty suggesting that these heifers entered precocious puberty. Percentage of heifers cycling over time by age and BW are presented in Figures 4 and 5, respectively. There was a treatment by age interaction on onset of puberty. Attainment of puberty is also thought to be correlated to body fat content (Zieba et al., 2004; Perry, 2011). In the current study there were no differences in plasma concentrations of leptin which are partially indicative of body fat deposition. However, circulating plasma cholesterol and fatty acids differed and may have played a larger role. Anderson et al. (2015d) also found similar results, but more research is necessary to confirm this speculation.

Conclusion

As originally hypothesized, increasing the inclusion rate of DDGS changed the metabolic profile. Total plasma fatty acids and PUFA were altered by dietary treatments, and there was a tendency for cholesterol to be altered. However, heifers maintained energy status without accumulating excess adipose tissue as indicated by plasma leptin concentrations. Plasma proportion and concentration of linoleic acid and arachidonic acid, which is a precursor for prostaglandins, increased as a result of increasing the dietary inclusion rate of DDGS. There was a treatment by age interaction on the onset of puberty; however, there were no differences in average age or BW at puberty. Overall, this research indicated that DDGS could be incorporated into growing dairy heifer limit-fed rations at up to 50% of dietary DM without causing negative effects on short or long-term energy status or onset of puberty.

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Table 9. Ingredients, nutrient composition of treatment diets, and nutrient intakes with increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay limit-fed to growing replacement Holstein dairy heifers

	Treatment[1]		
Item[2]	30DG	40DG	50DG
Ingredient, % DM			
DDGS	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
Nutrient[4], % of DM			
DM, %	86.7	86.7	86.8
CP	16.8	19.2	21.5
NDF	54.4	50.8	47.1
EE (Petroleum)	3.06	3.74	4.41
Starch	2.38	2.89	3.41
ME, Mcal/kg DM	2.27	2.39	2.51
NE_G, Mcal/kg DM	0.81	0.90	0.99
Nutrient intake, kg/d			
DM	6.49	6.21	5.84
CP	1.09	1.19	1.26
NDF	3.53	3.15	2.75
EE (Petroleum)	0.20	0.23	0.26
ME, Mcal/kg DM	14.7	14.8	14.7
NE_G, Mcal/kg DM	5.25	5.59	5.78

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

[4] % of DM, unless otherwise noted.

Table 10. Fatty acid composition of the grass hay and distillers dried grains with solubles (**DDGS**) used in the treatment diets limit-fed to growing Holstein dairy heifers

	Grass hay		DDGS	
Fatty acid[1]	Mean	SE	Mean	SE
	--------------------g/100g of fatty acid---------------------			
C10:0	5.23	0.835	0.81	0.052
C12:0	3.60	0.396	0.52	0.015
C12:1	10.4	0.745	0.70	0.012
C14:0	1.27	0.066	5.05	0.033
C16:0	7.36	0.301	12.5	0.060
C16:1	5.81	0.346	0.14	0.003
C18:0	0.78	0.066	1.79	0.008
C18:1, *cis* 11	1.35	0.135	17.3	0.047
C18:1, *trans* 11	0.10	0.036	0.74	0.003
C18:2, *cis* 9, *cis* 12	4.30	0.297	49.0	0.234
C18:3 ɤ	0.40	0.033	0.55	0.240
C20:0	18.2	1.033	4.76	0.042
C18:3 α	22.8	0.804	3.50	0.036
C18:2 *trans*[2]	1.46	0.086	0.17	0.004
C20:4	0.43	0.128	0.13	0.002
Others[3]	16.5	0.388	2.33	0.021
	-----------------------g/kg of DM--------------------			
C10:0	1.05	0.176	0.66	0.069
C12:0	0.72	0.076	0.42	0.006
C12:1	2.07	0.144	0.56	0.031
C14:0	0.25	0.015	4.06	0.151
C16:0	1.46	0.029	10.1	0.391
C16:1	1.16	0.088	0.11	0.005
C18:0	0.16	0.009	1.44	0.065
C18:1, *cis* 11	0.27	0.018	13.9	0.633
C18:1, *trans* 11	0.02	0.007	0.59	0.025
C18:2, *cis* 9, *cis* 12	0.86	0.056	39.3	1.528
C18:3 ɤ	0.08	0.004	0.46	0.203
C20:0	3.63	0.246	3.83	0.189
C18:3 α	4.56	0.313	2.81	0.113
C18:2 *trans*[2]	0.29	0.008	0.13	0.009
C20:4	0.09	0.027	0.11	0.005
Others[3]	3.29	0.033	1.87	0.079
Total	19.9	0.665	80.4	3.487

[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* 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 11. Fatty acid compositions of the treatment diets with increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay limit-fed to growing Holstein dairy heifers

	Treatment[1]					
	30DG		40DG		50DG	
Fatty acid[2], g/kg of DM	Mean	SE	Mean	SE	Mean	SE
C10:0	0.92	0.141	0.88	0.130	0.84	0.119
C12:0	0.62	0.051	0.59	0.043	0.56	0.035
C12:1	1.59	0.107	1.43	0.096	1.28	0.085
C14:0	1.39	0.043	1.77	0.058	2.15	0.074
C16:0	4.02	0.103	4.88	0.144	5.74	0.185
C16:1	0.83	0.061	0.72	0.053	0.62	0.044
C18:0	0.54	0.014	0.67	0.021	0.80	0.029
C18:1, *cis* 11	4.37	0.180	5.73	0.244	7.10	0.309
C18:1, *trans* 11	0.19	0.011	0.25	0.013	0.31	0.015
C18:2, *cis* 9, *cis* 12	12.4	0.44	16.2	0.59	20.1	0.75
C18:3 ɤ	0.19	0.060	0.23	0.081	0.27	0.101
C20:0	3.64	0.157	3.66	0.137	3.68	0.123
C18:3 α	3.96	0.234	3.79	0.210	3.61	0.187
C18:2 *trans*[3]	0.24	0.003	0.22	0.002	0.21	0.001
C20:4	0.09	0.020	0.09	0.018	0.10	0.016
Others[4]	2.82	0.045	2.68	0.050	2.53	0.055
Total	37.8	1.403	43.8	1.690	49.9	1.982

[1] 30% dietary inclusion rate of DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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, 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 12. Mean fatty acid intakes for Holstein heifers fed increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) 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	0.148	<0.01	<0.01	0.17	<0.01	0.89
C12:0	4.00	3.64	3.25	0.099	<0.01	<0.01	0.08	<0.01	0.90
C12:1	10.28	8.90	7.50	0.241	<0.01	<0.01	<0.01	<0.01	0.97
C14:0	9.02	10.99	12.56	0.307	<0.01	<0.01	<0.01	<0.01	0.60
C16:0	26.09	30.29	33.54	0.842	<0.01	<0.01	<0.01	<0.01	0.64
C16:1	5.37	4.48	3.61	0.122	<0.01	<0.01	<0.01	<0.01	0.98
C18:0	3.49	4.14	4.65	0.115	<0.01	<0.01	<0.01	<0.01	0.62
C18:1, *cis* 11	28.32	35.59	41.49	1.000	<0.01	<0.01	<0.01	<0.01	0.58
C18:1, *trans* 11	1.24	1.54	1.79	0.043	<0.01	<0.01	<0.01	<0.01	0.59
C18:2, *cis* 9, *cis* 12	80.36	100.77	117.33	2.832	<0.01	<0.01	<0.01	<0.01	0.58
C18:3 ɤ	1.25	1.43	1.57	0.040	<0.01	<0.01	<0.01	<0.01	0.66
C20:0	23.58	22.69	21.47	0.617	0.06	<0.01	0.98	0.02	0.83
C18:3 α	25.71	23.51	21.11	0.638	<0.01	<0.01	0.13	<0.01	0.90
C18:2 *trans*[3]	1.55	1.38	1.21	0.038	<0.01	<0.01	<0.01	<0.01	0.93
C20:4	0.59	0.58	0.55	0.016	0.30	<0.01	1.00	0.13	0.83
Others[4]	18.28	16.60	14.80	0.451	<0.01	<0.01	0.06	<0.01	0.90
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 DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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 13. Plasma fatty acid profile from wk 16 of the feeding period for Holstein heifers fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay in limit-fed rations

	Treatment[1]				*P-value*[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 ɤ	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* ɤ	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
Saturated	37.23	38.47	36.87	0.470	0.05	0.59	0.02

[1] 30% dietary inclusion rate of DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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 distillers dried grains with solubles (**DDGS**) in replacement of grass hay in limit-fed rations

	Treatment[1]				*P-value*[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 *cis* 11, *cis* 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
Saturated	515.83	528.32	567.79	34.984	0.09	0.03	0.52

[1] 30% dietary inclusion rate of DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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 increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay in limit-fed rations

	Treatment[1]				*P value*[2]				
Item	30DG	40DG	50DG	SEM	Trt	Wk	Trt× wk	L	Q
Cholesterol, mg/dL	93.48	89.15	97.13	2.96	0.17	<0.01	0.39	0.39	0.10
Glucose[3], mg/dL	76.26	77.74	77.33	1.67	0.81	0.10	0.88	0.65	0.65
IGF-1, ng/mL	102.7	100.0	109.4	4.27	0.29	<0.01	0.30	0.27	0.25
Insulin, ng/mL	1.05	1.12	1.15	0.099	0.78	<0.01	0.61	0.50	0.84
Leptin, ng/mL	4.42	4.35	4.59	0.091	0.19	0.14	0.57	0.22	0.18
Plasma urea nitrogen, mg/dL	17.83	17.82	19.90	0.495	<0.01	<0.01	0.90	<0.01	0.09
Triglycerides, mg/dL	17.82	19.14	18.47	0.643	0.36	0.89	0.54	0.48	0.21

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

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

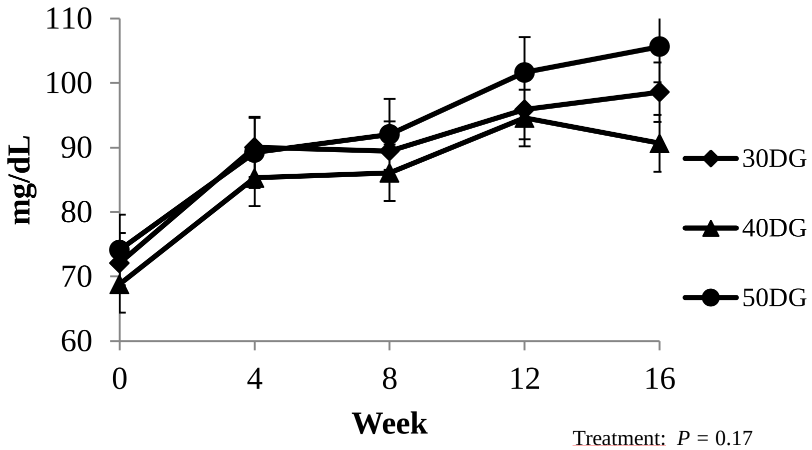
[3] Glucose was measured from serum samples instead of plasma.

Table 16. Mean age and body weight at puberty for Holstein heifers fed distillers dried grains with solubles (**DDGS**) in replacement of grass hay in 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

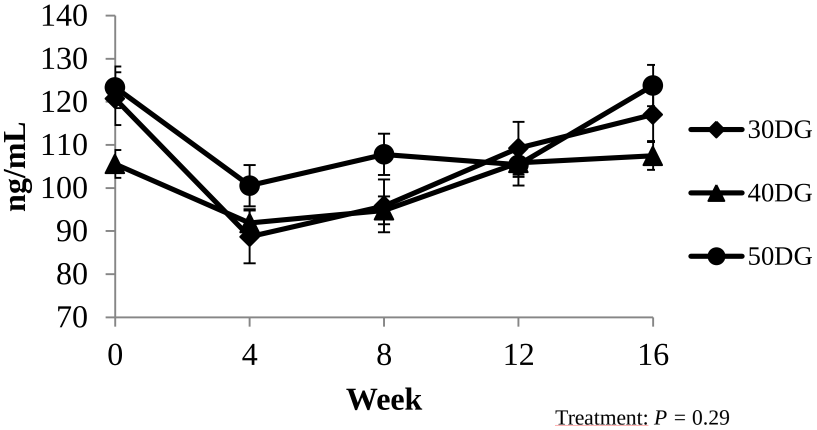
[1] 30% dietary inclusion rate of DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**50DG**).

Figure 1. Cholesterol concentrations of Holstein heifers limit-fed treatment diets with increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay



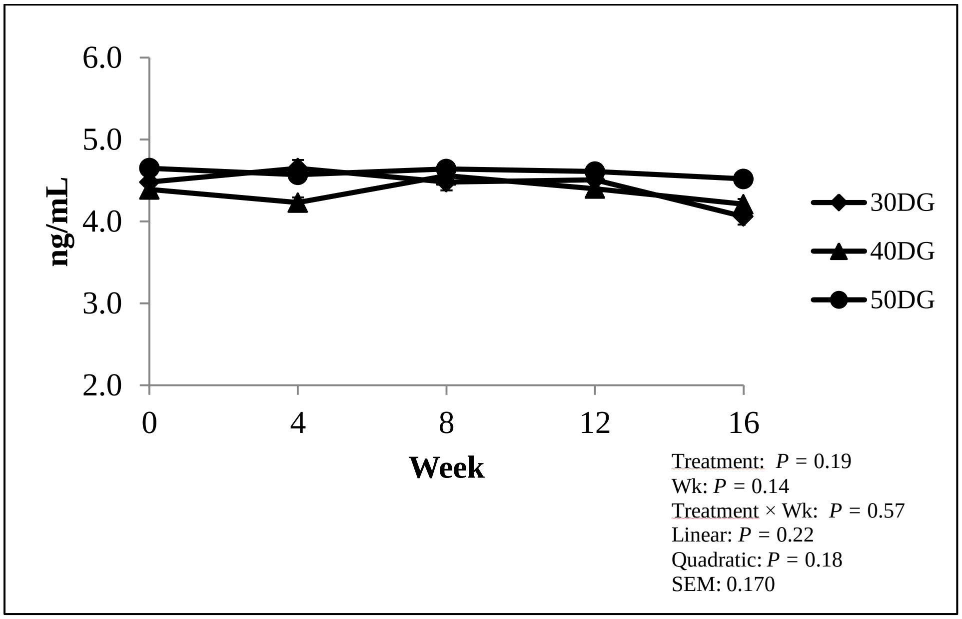
Treatment: *P* = 0.17
Wk: *P* < 0.01
Treatment × Wk: *P* = 0.39
Linear: *P* = 0.39
Quadratic: *P* = 0. 10
SEM: 3.72

Figure 2. Insulin-like growth factor -1 (**IGF-1**) concentrations of Holstein heifers limit-fed treatment diets with increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay



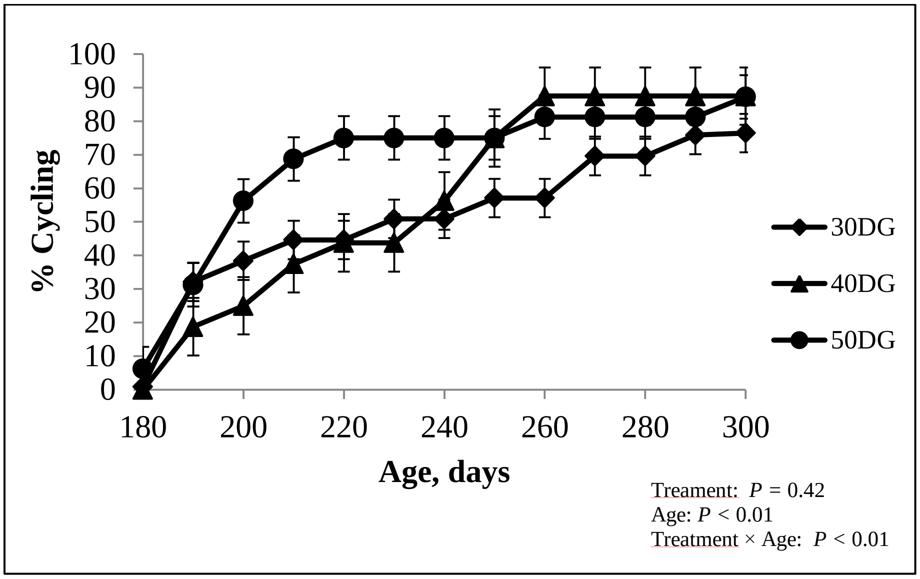
Treatment: $P = 0.29$
Wk: $P < 0.01$
Treatment × Wk: $P = 0.30$
Linear: $P = 0.27$
Quadratic: $P = 0.25$
SEM: 5.68

Figure 3. Leptin concentrations of Holstein heifers limit-fed treatment diets with increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay



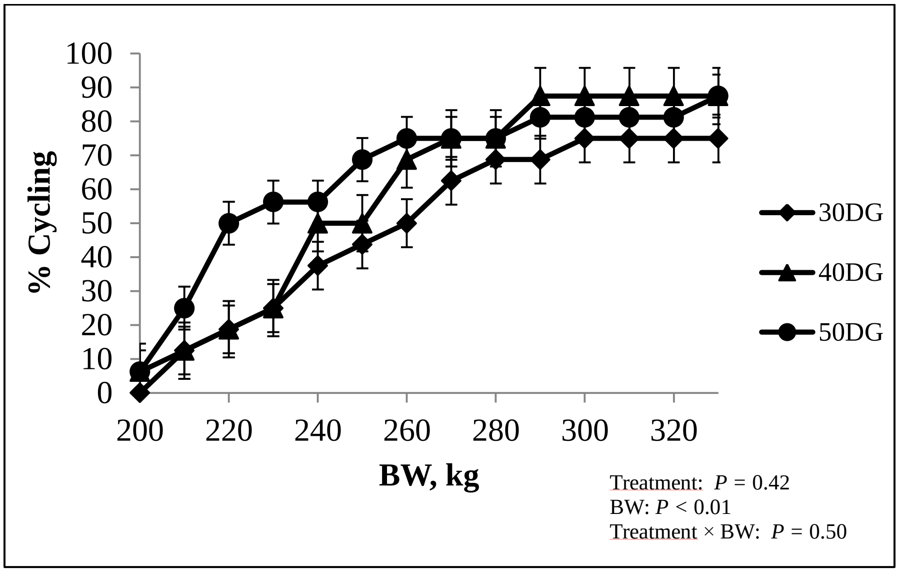
Treatment: *P* = 0.19
Wk: *P* = 0.14
Treatment × Wk: *P* = 0.57
Linear: *P* = 0.22
Quadratic: *P* = 0.18
SEM: 0.170

Figure 4. Percent of Holstein heifers pubertal (cycling) by age that were limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay



Treament: $P = 0.42$
Age: $P < 0.01$
Treatment × Age: $P < 0.01$

Figure 5. Percent of Holstein heifers pubertal (cycling) by body weight (**BW**) that were limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay



Treatment: $P = 0.42$
BW: $P < 0.01$
Treatment × BW: $P = 0.50$

CHAPTER 4:

FEEDING DISTILLERS DRIED GRAINS IN REPLACEMENT OF FORAGE IN LIMIT-FED DAIRY HEIFER RATIONS: EFFECTS ON POST TRIAL PERFORMANCE

Abstract

The objective of this study was to determine the effect of increasing the inclusion rate of distillers dried grains (**DDGS**) in replacement of forage in limit-fed diets on the long-term reproductive and lactation performance of dairy heifers. A 16-wk randomized complete block design study was conducted using 48 Holstein heifers (199 ± 2 d of age) with three treatments. Treatments were 1) 30% DDGS (**30DG**), 2) 40% DDGS (**40DG**), and 3) 50% DDGS (**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. After completing the feeding study heifers were fed a common diet according to standard herd management. Data on reproductive performance and milk production for the first three months of lactation were collected for each heifer from dairy herd records. At 3 wk prepartum and at calving, BW, frame measurements, and body condition score (**BCS**) were recorded. There were no treatment by wk interactions for any of the reproductive or frame measurements recorded. There was a linear tendency for age at first service to decrease with increasing amounts of DDGS; however, there were no differences in any other reproductive or frame measurements. There was a treatment by wk effect for somatic cell count (**SCC**); however, there were no other differences for any of the lactation parameters measured. Results demonstrate that up to 50% of diet can be fed as DDGS to peripubertal dairy heifers without negative consequences to long-term performance.

Keywords: distillers grains, heifer, lactation performance

Introduction

The optimal growth rate and feeding strategy of growing dairy heifers in which to maximize reproductive and lactation performance has been well researched. Increasing ADG in order to shorten the length of the rearing period and decreasing age at first calving has been shown to result in an earlier return on investment (Ettema and Santos, 2004). However, increasing the ADG of growing dairy heifers has been demonstrated to have a negative impact on mammary development and lactation performance (Van Amburgh et al., 1998; Zanton and Heinrichs, 2005; Meyer et al., 2006a).

Feeding heifers high concentrate diets, but restricting ADG during the prepubertal period has been demonstrated to maintain milk production when compared to high forage diets (Carson et al., 2000; Zanton and Heinrichs, 2009). Chapter 2 demonstrated limit-feeding diets with increasing inclusion amounts of DDGS and found no differences in growth performance or ADG. Anderson et al., (2015c) limit-fed heifers a corn and soybean product based control diet, low-fat DDGS, or high-fat DDGS and found that heifers fed the DDGS diets had similar or improved milk production.

There has been very limited research examining the effect of limit-feeding diets with DDGS as the primary concentrate ingredient during the prepubertal growth period of dairy heifers on subsequent reproductive and lactation performance. Therefore, the main objective of this research was to evaluate the effect of increasing the inclusion rate of DDGS in replacement of forage in limit-fed diets on the post trial reproductive and first lactation performance of dairy heifers. It was hypothesized that increasing the inclusion rate of DDGS would result in a younger age at first service and maintained or improved lactation performance.

Materials and Methods

Experimental Design

Forty-eight Holstein heifers (199 ± 2 d of age) were originally used in a randomized complete block design with three treatment diets. The feeding period lasted for 16 wk, beginning during the prepubertal period. Treatment diets (Table 17) were: 1) high forage with 30% of diet as DDGS (**30DG**), 2) moderate forage with 40% of diet as DDGS (**40DG**), and 3) low forage with 50% of diet as DDGS (**50DG**) on DM basis. The forage portion of the diets consisted of grass hay. The amount of feed offered was determined as a percentage of BW and decreased with increasing concentrations of DDGS in order to allow for 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).

Diets were formulated using the NRC (2001) to provide similar energy intakes when fed to a 250 kg BW Holstein heifer. Heifers were fed individually using a Calan gate feeding system (American Calan Inc., Northwood, NH). Nutrient composition and average intakes are also provided in Table 17. Details regarding diet formulation and nutrient analysis are described in Chapter 2. During the feeding period, growth performance, rumen fermentation, nutrient digestibility, metabolic profile, and onset of puberty were evaluated. After the feeding period, heifers were returned to the general herd at the South Dakota State University Dairy Research and Training Facility (SDSU-DRTF; Brookings, SD). Heifers were then managed under standard farm operating procedures.

Data and Measurement Collection

Data on reproductive performance which included the age at first artificial insemination (**AI**) service, number of AI services, and age at conception were collected from herd health records. Age at conception was based on when pregnancy was confirmed. Body growth measurements including BW, withers and hip heights, heart and paunch girth, body length, and hip width were measured one day 3 wk prepartum (based on predicted calving dates) at approximately 4 h post-feeding. 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 by two individuals based on the scale described by Wildman et al. (1982) with 1 = emaciated and 5 = obese. Within 48 h post-calving, heifers were once again weighed and measured are previously described. Calf weights were also recorded. Because of the staggered dates that heifers were brought on to the prepubertal feeding trial due to heifer availability and the differing amounts of time that it took for heifers to conceive, calving took place over a fifteen month period from January 2015 to February 2016.

Lactation performance data was collected from January 2015 through June 2016. Data was collected from Dairy Herd Improvement Association (**DHIA**) records on each individual heifer for the first three months of lactation. Cows were milked twice daily at 0600 and 1800 h. For statistical analysis, data were analyzed by month of lactation because milk samples were collected for DHIA analysis randomly during each month from the farm, and calving dates differed for each heifer, the days in milk (**DIM**) at each test date were not equal for each heifer. Milk samples were analyzed for fat and protein concentration, as well as somatic cell count (**SCC**) at Heart of America DHIA Laboratory

(Manhattan, KS). Mid-infrared spectroscopy (Bently 2000 Infrared Milk Analyzer, Bently Instruments, Chaska, MN; AOAC, 2002) was used for the analysis of fat and protein content. A flow cytometer laser (Somacount 500, Bently Instruments: AOAC, 2002) was used for SCC. Energy corrected milk (**ECM**) was calculated as: ECM = [(0.327 × kg milk) + (12.95 × kg fat) (7.2 × kg protein)] (Orth, 1992).

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The MIXED procedures of SAS were used for the analysis reproductive, BW, and frame measurement data. The model included treatment with block included as a random variable because samples were analyzed from a single time period. Body weight and frame measurements taken 3 wk prepartum were analyzed separately from BW and frame measurements taken at calving.

Lactation performance data were analyzed as a randomized complete block design with month as the repeated measure and the term heifer (block) as the subject using the PROC MIXED procedures of SAS (Littell et al., 2006). The model included treatment, month, and treatment × month interactions. Akaike's criterion was used to determine the most suitable covariance structure in repeated measures for each parameter. Covariance structures tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. Compound symmetry resulted in the least absolute Akaike's values and was used for the final model. 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.

Results and Discussion

Reproductive Performance

Reproductive performance, as well as body weight and frame measurements 3 wk prepartum and at parturition are presented in Table 18. Age at first service tended to linearly decrease with increasing inclusion amount of DDGS. According to Chapter 3, heifers on the 50DG treatment tended to have increased concentrations of plasma cholesterol. This is of interest because cholesterol is a precursor to steroid hormones such as progesterone (Talavera et al., 1985). Additionally, a large percentage of heifers on the 50DG treatment were cycling at an earlier age. Anderson et al. (2015b) found heifers to be younger at age at first service due to a difference in farm management at the time that the study was conducted.

There was a quadratic effect for hip height at parturition with heifers on the 30DG and 50DG treatments having greater hip heights than 40DG heifers. There was also a linear decrease in body length at parturition with increasing inclusion amounts of DDGS. However, these differences are numerically small. There were no other differences in reproductive or growth parameters or calf weight among treatments, demonstrating that prepubertal diets had minimal effect on post-trial performance. According to Hoffman (1997) Holstein heifers should be between 580 and 635 kg at calving. Heifers on the current experiment were within these guidelines. Reproductive and body size parameters were similar to those reported by Anderson et al., (2015c).

Percent conception based upon artificial insemination service number is presented in Figure 6. There were no significant differences among treatments, and heifers had approximately 40% conception on first service. The limited number of heifers in the current experiment may explain the lack of statistical differences among treatments. Therefore, additional research is warranted to further understand the interaction of the increasing inclusion rate of DDGS and reproduction.

Lactation Performance

Lactation performance during the first three months of lactation is presented in Table 19. There was a treatment by wk interaction for SCC. The reason for this is unknown because there has not been a demonstrated interaction between feeding strategy or ADG on SCC. There were no differences in any of the other lactation parameters measured. During the prepubertal period, heifers had ADG of 0.91, 0.96, and 0.95 kg/d. This is greater than the recommended ADG of 0.8 kg/d to maximize lactation milk production (Zanton and Heinrichs, 2005). However, there were no differences in production among treatments in the current experiment. Anderson et al. (2015c) demonstrated an increase in milk production for heifers limit-fed low- or high-fat DDGS compared to a control diet with similar ADG among treatments. However, the ADG was also greater than recommended (Anderson et al., 2015c). This suggests that heifers fed DDGS were able to achieve similar or improved mammary parenchyma development. It also suggests that form of dietary energy (starch versus fat) may play a role in growth and development during the prepubertal period (Anderson et al., 2015c). In the current experiment there appears to be less of an effect of inclusion amount of DDGS fed during the prepubertal period on milk production.

Conclusion

In agreement with our hypothesis, limit-feeding diets containing increasing amount of DDGS at up to 50% of dietary dry matter during the prepubertal period decreased age at first service while resulting in minor differences in frame measurements at parturition. Heifers fed DDGS at 50% of diet dry matter also maintained lactation performance compared to heifers fed DDGS at 30 and 40% of diet dry matter. This indicates that heifers can be limit-fed increased amounts of DDGS during the prepubertal period without detrimental effects on post pubertal growth, reproduction, or lactation performance during the first three months of the first lactation.

Table 17. Ingredient and nutrient composition of treatment diets with increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay limit-fed to growing replacement Holstein dairy heifers during the prepubertal growth phase

	Treatment[1]		
Item	30DG	40DG	50DG
Ingredient[2], % DM			
DDGS	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
Nutrient, % of DM			
DM, % of diet	86.7	86.7	86.8
CP	16.8	16.8	16.8
Ether extract (diethyl)	5.17	5.17	5.17
Starch	2.38	2.38	2.38
ME, Mcal/kg	2.38	2.38	2.38
NE_G, Mcal/kg	2.89	2.89	2.89

[1] 30% dietary inclusion rate of DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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 18. Reproductive performance, body weight, and frame measures for Holstein heifers limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay during the prepubertal growth period

	Treatment[1]				*P-value*[2]		
Item	30DG	40DG	50DG	SEM	Trt	L	Q
Age at first service, d	411.5	413.6	399.0	5.13	0.09	0.08	0.19
Age at conception, d	412.6	413.3	434.8	25.8	0.51	0.31	0.59
AI service, no.	1.80	1.61	2.17	0.75	0.52	0.45	0.39
Predicted age at calving, d	697.7	681.4	711.9	24.35	0.34	0.51	0.19
Actual age at calving, d	698.2	682.9	715.2	25.1	0.34	0.44	0.20
Body measures, 3 wk prepartum							
BW, kg	681.3	667.1	693.7	18.8	0.57	0.64	0.34
Withers height, cm	149.2	148.6	150.2	1.36	0.38	0.54	0.21
Hip height, cm	150.3	149.4	150.9	0.92	0.48	0.65	0.26
Heart girth, cm	199.9	201.2	202.1	5.45	0.83	0.54	0.96
Paunch girth, cm	243.5	246.1	246.5	2.51	0.63	0.39	0.70
Body length, cm	154.6	154.6	154.0	1.14	0.90	0.69	0.82
Hip width, cm	55.8	55.9	56.3	0.60	0.82	0.55	0.84
BCS	3.29	3.32	3.33	0.074	0.73	0.44	0.89
Body measures, at parturition							
BW, kg	631.6	615.3	623.7	29.5	0.74	0.71	0.50
Withers height, cm	149.6	147.8	149.8	2.12	0.30	0.87	0.13
Hip height, cm	151.9	149.8	152.5	0.96	0.10	0.66	0.04
Heart girth, cm	201.5	200.3	201.3	5.83	0.94	0.96	0.73
Paunch girth, cm	237.2	238.0	234.0	2.59	0.50	0.39	0.43
Body length, cm	157.9	154.9	153.8	1.13	0.04	0.02	0.48
Hip width, cm	55.3	55.2	55.0	0.60	0.93	0.71	0.89
BCS	3.16	3.19	3.19	0.070	0.88	0.71	0.73
Calf BW, kg	39.2	40.4	39.3	2.77	0.78	0.95	0.48

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

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

Table 19. Milk production performance based on Dairy Herd Improvement Association (**DHIA**) records for Holstein heifers limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay during the prepubertal growth period

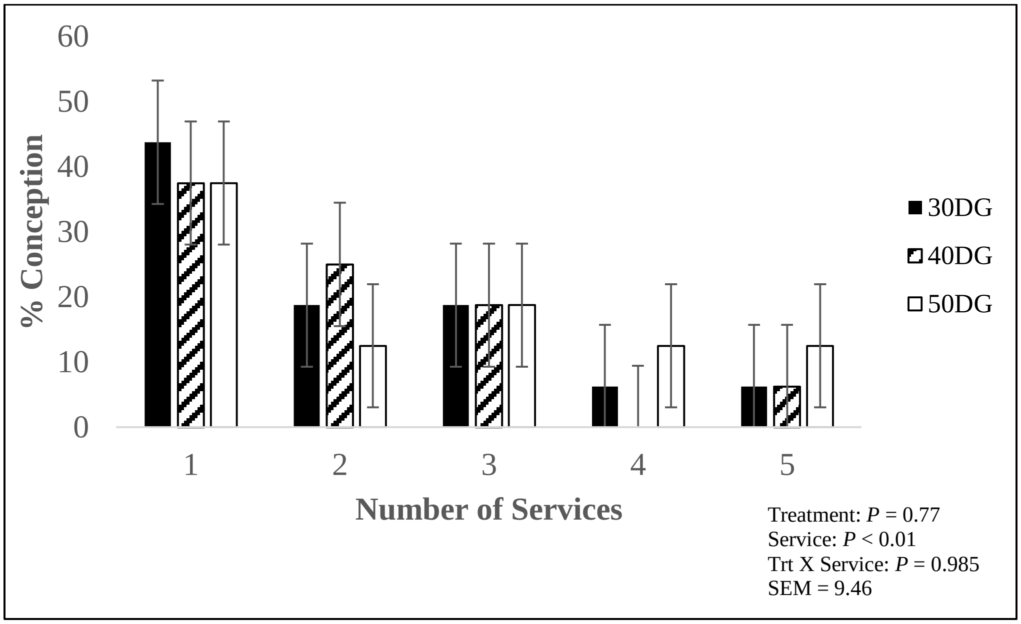
	Treatment[1]				*P-value*[2]				
Item	30DG	40DG	50DG	SEM	Trt	mo	Trt*mo	L	Q
Milk yield, kg	27.4	28.8	29.4	1.85	0.74	<0.01	0.30	0.46	0.84
ECM[3], kg	19.3	19.8	20.2	1.17	0.84	0.03	0.40	0.56	0.93
Fat, %	4.54	4.66	4.66	0.29	0.94	<0.01	0.61	0.76	0.85
Fat yield, kg/d	0.59	0.59	0.60	0.040	0.99	0.96	0.40	0.90	0.93
Protein, %	2.88	2.92	2.96	0.08	0.80	0.07	0.92	0.51	0.96
Protein yield, kg/d	0.36	0.38	0.39	0.024	0.65	<0.01	0.24	0.36	0.97
Somatic cells, × 10^3/mL	451.0	132.6	113.4	84.0	0.01	0.06	0.02	<0.01	0.12

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

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

[3] ECM = [(0.327 × kg of milk) + (12.95 × kg of fat) + (7.2 × kg of protein)] (Orth, 1992).

Figure 6. Percent conception based on service number for Holstein heifers limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay during the prepubertal growth period



Treatment: $P = 0.77$
Service: $P < 0.01$
Trt X Service: $P = 0.985$
SEM = 9.46

CHAPTER 5:

GROWTH PERFORMANCE, RUMEN FERMENTATION, NUTRIENT UTILIZATION, AND METABOLIC PROFILE OF HEIFERS LIMIT-FED DISTILLERS DRIED GRAINS WITH AD LIBITUM FORAGE

Abstract

The objective of this study was to determine the effects of feeding a corn and soybean product based concentrate mix or distillers dried grains with solubles (**DDGS**) concentrate mix with ad libitum grass hay to dairy heifers. A 16-wk randomized complete block design study was conducted using 24 heifers (18 Holstein and 6 Brown Swiss; 219 ±2 d of age; 230 ± 4k kg BW) to evaluate the effect of diet on dry matter intake (**DMI**), growth performance, rumen fermentation, metabolic profile, and nutrient digestibility. Treatments were 1) corn and soybean product concentrate mix (**CON**), and 2) DDGS based concentrate mix (**DDG**). Both concentrate mixes were limit-fed at 0.8% of body weight (**BW**) and grass hay was offered ad libitum. Heifers were individually limit-fed using Calan gates and orts were recorded daily at feeding. Heifers were weighed every 2 wk and ration concentrate mix offered was adjusted accordingly. Frame measurements and body condition score (**BCS**) were recorded every 2 wk. Rumen fluid was collected via esophageal tubing during wk 12 and 16 for pH, ammonia N, and volatile fatty acids (**VFA**) analysis. Jugular blood samples were collected every 4 wk for metabolite and metabolic hormone analysis. Total tract digestibility of nutrients was evaluated during wk

16 by fecal grab sampling. There were no treatment by wk interactions for any of the growth parameters measured and growth parameters as well as DMI did not differ between treatments. There was a treatment by time interaction for butyrate percentage with heifers fed DDG having a greater percentage. Acetate concentration, total VFA concentration, acetate molar percentage, and acetate: propionate decreased with the DDG treatment, while propionate molar percentage increased. There were no treatment by wk interactions for any of the metabolites or metabolic hormones measured. There was a tendency for glucose and plasma urea nitrogen concentration to decrease with DDG. Plasma cholesterol and insulin increased with DDG. Results demonstrated that limit-feeding heifers DDGS at 0.8% of BW with ad libitum grass hay maintained growth performance, ADG, DMI, gain: feed, with shifts in the metabolic profile compared to the corn and soybean product concentrate mix.

Keywords: distillers grains, dairy heifer, growth performance

Introduction

Previous research has demonstrated that distillers dried grains with solubles (**DDGS**) can improve gain: feed and maintain growth performance and average daily gain (**ADG**) when limit-fed or fed ad libitum in a total mixed ration (**TMR**) to growing dairy heifers (Schroer et al., 2014; Anderson et al., 2015a; Chapter 2, 3, and 4). However, there is relatively little research that has focused on limit-feeding a DDGS based concentrate mix compared to a corn and soybean product based concentrate mix with ad libitum grass hay.

Feeding high fiber feedstuffs to growing dairy heifers may decrease diet digestibility (Zanton and Heinrichs, 2008). Reducing dry matter intake (**DMI**) could also lead to improved digestibility of nutrients. Anderson et al. (2015a) demonstrated that feeding DDGS improved crude protein (**CP**), neutral detergent fiber (**NDF**), acid detergent fiber (**ADF**) digestibility when compared to a corn and soybean product based control concentrate mix in limit-fed diets. However, the forage was also limit-fed in these diets. In limit-fed diets, DDGS been compared to corn and soybean product based concentrate mixes and has been demonstrated to maintain growth performance and improve nutrient utilization in growing dairy heifers (Anderson et al., 2015a). However, there is very limited research that has examined the effect of limit-feeding a corn and soybean product based concentrate mix and DDGS based concentrate mix with ad libitum forage.

Therefore, the main objective of this study was to determine the effect of limit-feeding a corn and soybean product based concentrate mix compared to DDGS with ab libitum grass hay and determine its effects on DMI, growth, rumen fermentation, metabolic profile, and nutrient digestibility. We hypothesized that heifers fed DDGS would have improved gain: feed because of a slightly greater dietary fat concentration causing the heifers to eat less hay, but growth performance would be maintained.

Materials and Methods

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

Experimental Design

Twenty-four heifers (18 Holstein and 6 Brown Swiss; 219 ± 2 d of age; 230 ± 4 kg BW) were used in a randomized complete block design with two treatment diets. Heifers were blocked in groups of two, based on breed, birth date, and BW. Heifers were randomly assigned to treatment within blocks. Heifers were added to the study based on farm calving rates and were introduced in multiples of six with a target start age of 7 months. Heifers were acclimated to the barns and feeding system for approximately two wk followed by an experimental feeding period of 16 wk.

Treatment diets (Table 20) were: 1) corn and soybean product concentrate mix (**CON**), and 2) DDGS based concentrate mix (**DDG**). Both concentrate mixes were limit-fed at 0.8% of BW (DM basis) and grass hay was fed ad libitum. Diets were formulated using the NRC (2001) to meet a target ADG of 0.8 kg/d when fed to a 250 kg BW Holstein heifer and to provide similar protein and energy intakes. The 250 kg BW was a pre-estimated average BW for heifers during the study based on age and herd data. On the last two d of each two wk interval, heifers were weighed and then amount of feed offered was determined for the next two wk. Amount of each concentrate mix offered was also adjusted using DM analysis of feedstuffs.

In order to avoid variation in production within plant and over time, DDGS was purchased in one batch and stored at the South Dakota State University Dairy Research and Training Facility. The corn and soybean product concentrate mix was mixed in one ton batches at the South Dakota State University feed mill as needed throughout the feeding period. Hay was purchased in one batch.

Animal Care and Feeding

This study was conducted at the South Dakota State University Dairy Research and Training Facility (**SDSU DRTF**; Brookings, SD). The study was completed from March 2015 through September 2015 to accommodate available animals and pen space. Heifers were observed daily for health problems and treated according to routine management practices at the DRTF.

Heifers were housed in pens of 6 heifers each. Each pen had an inside roofed area (7 m × 4 m) and an outside dirt exercise lot (7 m × 23.5 m). The inside areas of the pens were a bedded pack, and were bedded with straw once every 2 wk. Because the consumption of bedding material can be a concern when limit-feeding, pens were only bedded once every 2 wk. Each pen was provided with water ad libitum. Heifers were fed once daily at 0830 h using the Calan gate feeding system (American Calan Inc., Northwood, NH) and individual intakes were measured. Heifers that had consumed most of their hay during the day were offered additional hay at 1700 h. Bales of hay were coarsely pre-ground with a vertical tub grinder to ease feeding. Diet components were individually weighed for each heifer. The mineral mix was mixed with the concentrate mix before mixing with the grass hay. Orts were weighed and recorded every morning before feeding. Samples of the concentrate mixes and grass hay were taken each wk and stored at -20°C until analysis. Ort samples from each group were collected and composited by treatment each week. Individual ingredient samples from the corn and soybean product concentrate mix were taken each time a batch was mixed.

Animal Measurements and Sampling

Body growth measurements including BW, withers and hip heights, heart and paunch girth, body length, and hip width were measured on 2 consecutive d approximately 4 h post-feeding at the beginning of the study and then every 2 wk 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 wk 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.

Rumen fluid was sampled from each heifer on 2 consecutive d during wk 12 and 16 at approximately 4 h 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 pH meter (Waterproof pH Testr 30, Oakton Instruments, Vernon Hills, IL) and 2 aliquots (10 mL) 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.

Blood samples were taken on two consecutive days during wk 0, 4, 8, 12, and 16 of the feeding study for the analysis of cholesterol, glucose, insulin, plasma urea nitrogen, and triglycerides. Blood samples were taken approximately 4 h post-feeding (1230 h) via venipuncture of the jugular vein into vacutainer tubes (Becton, Dickinson, and Company, Franklin Lakes, NJ) containing sodium fluoride (NaFl) and potassium oxalate (K 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 h of collection. 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 for metabolites or hormones, plasma or serum from the two consecutive days during each of the blood sampling weeks (wk 0, 4, 8, 12, and 16) were both analyzed and then averaged for statistical analysis.

For analysis of total tract digestibility, fecal samples were collected during wk 16 of the feeding period. Acid detergent insoluble ash (**ADIA**) was used as an internal digestibility marker. Individual heifer orts and fecal grab samples were collected during 3 consecutive d in wk 16 and stored at - 20°C until processing and analysis. Fecal sampling time points were scheduled so that the samples represented every 3 h in a 24 h feeding cycle.

Laboratory Analysis

Feed samples were dried for 24 h at 105°C for DM analysis in order to adjust dietary ingredient inclusion rates and determine DMI. Samples of the control concentrate, DDGS concentrate mix, and grass hay were collected once weekly and frozen at -20°C until analysis. Samples of the corn and soybean products for the control concentrate mix, DDGS, and grass hay were thawed and samples from 4 consecutive wk were composited on an as-fed basis by weight. Composite samples were dried in duplicate for 48h 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. Philadelphis, 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 h at 450°C in a muffle furnace (AOAC 17th ed., method 942.05; 2002). 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. Petroleum ether was used 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 the corn and soybean product concentrate mix, DDGS, and grass hay were further composited into three month composites. Group ort samples were also further composited into four month composites. Samples were sent to a commercial laboratory (Dairyland Laboratories, Inc. Arcadia, WI) for analysis of minerals (Ca, Cl, Mg, P, K, Na, S) and starch. 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).

Rumen fluid samples preserved with sulfuric acid were thawed and centrifuged at 30,000 × g for 20 minutes at 4°C (Centrifuge: Eppendorf 5403, Eppendorf North America, Hauppauge, NY) and analyzed for ammonia N using a colorimetric assay performed 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 at 4°C and analyzed for acetate, propionate, butyrate, isobutyrate, isovalerate, and valerate concentrations 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.

Metabolites (cholesterol, glucose, plasma urea nitrogen, and triglycerides) were analyzed with commercially available enzymatic or colorimetric assay kits 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.

Insulin was analyzed using a commercially available insulin assay (MP Biomedical) according to the manufacturer's directions. Increasing volumes of bovine serum (25, 50, 75, and 100, μL) produced a displacement curve that was parallel (P = 0.60) to the standard curve (Slope = 1.93 ± 0.22 for standard curve; Slope = 1.77 ± 0.20 for bovine serum). Addition of known amounts of insulin (35 and 155 μIU/mL) to cow serum were accurately recovered (106%). Interassay and intraassay coefficient of variation was 10.10% and 3.85% respectively, and assay sensitivity was 5.5 μIU/mL.

Fecal 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.

Orts were collected each day during the collection period. Orts were composited based on proportions of weight from each day. 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 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 (2002). Digestibility calculations were determined according to Merchen (1988).

Statistical Analysis

All data were analyzed using SAS version 9.4 (SAS Institute Inc., Cary, NC). The MEANS procedure of SAS was used to estimate the means and standard errors of the nutrients of the monthly feed composites.

Heifer intake, growth data, nutrient intake, rumen fermentation, and plasma metabolites and hormone parameters were analyzed as a randomized complete block design with week as the repeated measure and the term heifer (block) as the subject using the PROC MIXED procedures of SAS (Littell et al., 2006). The model included treatment, wk, breed, as well as treatment × wk, treatment × breed, and treatment × breed × week interactions. Initial body size measurements, BW, and metabolite concentrations were included as covariates within the model. Akaike’s criterion was used to determine the most suitable covariance structure in repeated measures for each parameter. Covariance structures tested were compound symmetry, first-order autoregressive, Toeplitz, and unstructured. Compound symmetry resulted in the least absolute Akaike’s values and was used for the final model. Significant differences among treatments were declared at $P \leq 0.05$ and tendencies were declared at $0.05 < P \leq 0.10$

Regression procedures of SAS were used to determine average change per day for ADG and body frame measurements. The *P-values* for the interaction of treatment and time using MIXED analysis were used to determine significance of change per day among treatments (Kutner et al., 2004). Gain to feed ratio was calculated as the ratio of ADG (slope of BW regression) to DMI for each treatment. For comparison of analyses, ADG and gain: feed were also calculated based on 2 wk interval data and analyzed using MIXED procedures with repeated measures similar to frame size parameters.

The MIXED procedures of SAS were used for the analysis of total tract digestibility of nutrients. The model included treatment with block included as a random variable because samples were analyzed from a single time period.

Results and Discussion

Feed Analysis

The nutrient composition of the individual ingredients used in the corn and soybean product concentrate mix is presented in Table 21. Values are comparable to those listed in the NRC (2001) for the same feedstuffs. Nutrient composition of the CON and DDG concentrate mixes, as well as the grass hay is presented in Table 22. Because the DDGS was purchased in one batch at the beginning of the study, there was little variation in the nutrient composition over the duration of the study; however, there was some variation in the nutrient composition of the grass hay. Nutrient composition of the CON concentrate mix also varied very little.

Average nutrient composition of the experimental diets over the duration of the study is presented in Table 23. The nutrient composition was calculated based upon average intake of concentrate and grass hay nutrients and the nutrient composition of the monthly group ort samples for each treatment. Average EE, NDF, and ADF composition were greater for the DDG diet; whereas NFC and starch were decreased in the DDG diet. Because heifers were limit-fed the concentrate mix and given grass hay ad libitum, there was some variation in the nutrient composition of the rations over time; however, rations were providing adequate nutrients to the heifers and are comparable to those typically fed to growing dairy heifers.

Differences in the nutrient composition of the rations were affected by the nutrient intakes (Table 24). Neutral detergent fiber intake tended to increase and EE increased with DDGS due to the increased concentration of NDF and EE in the DDG concentrate mix compared to the CON. Starch and NFC intake decreased with the DDG concentrate mix due to decreased concentrations of these nutrients in the DDG concentrate mix.

Heifer Performance

Body weight, DMI, and gain: feed results are presented in Table 25. There were no interactions of treatment by week for any of the parameters measured. There were also no differences in BW, ADG, or gain: feed. However, the ADG in this experiment was greater than the target recommendation of 0.8 kg/d (Zanton and Heinrichs, 2005). This experiment, as well as Anderson et al. (2015a) and Chapter 2 suggest that the NRC (2001) overestimates the energy requirements of growing dairy heifers or underestimates the energy provided by ingredients. Dry matter intake was expected to decrease and gain: feed was expected to be improved with the DDG diet; however, this was not observed.

Frame measurements are presented in Table 26. Based on genomic data, Holstein heifers had similar predicted transmitting ability for type composite score (1.49 and 1.57 for CON and DDG, respectively, SEM = 0.154, $P = 0.72$); as a result, it was not used as a covariate term for growth performance. There were no treatment by week interactions for any of the growth parameters measured. Frame measurements increased over time, but were not different among treatments. There was also no difference in change per day for any of the frame measurements, suggesting that heifers were consuming adequate nutrients to promote growth throughout the experimental period. There was also no difference in BCS (Table 26). These results are consistent with those found by Anderson et al. (2015a) and Chapter 2 who limit-fed total rations containing DDGS to growing dairy heifers.

Rumen Fermentation

Rumen fermentation characteristics are presented in Table 27. There was a tendency for a treatment by week interaction for butyrate molar percentage. Acetate concentration and molar percentage, total VFA concentration, and acetate: propionate decreased, while propionate molar percentage increased with the DDG diet compared to the CON diet. The shift in molar VFA concentrations is most likely the result of the difference in starch concentration between the concentrate mixes. This may have also led to the decrease in acetate concentration and molar percentage and increase in propionate molar percentage in the DDG diet. This suggests that heifers fed the DDG diet had more efficient rumen fermentation as demonstrated by greater propionate molar percentage because there is less methane and carbon dioxide production in propionate as compared to acetate (Fahey and Berger, 1988). However, this shift in rumen fermentation did not result in differences in DMI or gain: feed in the DDG diet.

Metabolites and Metabolic Hormones

Average metabolite and metabolic hormone concentrations are presented in Table 28. There were no treatment by week interactions for any of the metabolites or metabolic hormones measured. There were no differences in concentrations of plasma triglycerides which are the storage form of fat within the body. This is consistent with results reported in Chapter 3 in which there were no differences in concentrations of plasma triglycerides when heifers were fed increasing concentrations of DDGS in replacement of forage. However, Park et al. (1983) found that increasing the dietary fat by increasing the inclusion rate of sunflower seeds led to an increase in the concentration of plasma triglycerides. There was an increase in the concentration of plasma cholesterol with the DDG diet compared to the CON diet. This is speculated to be a result of the increased dietary fat from the DDGS in the DDG diet. Anderson et al. (2015b) limit-fed dairy heifers a corn and soybean product based concentrate diet, low-fat DDGS, and high-fat DDGS diet and found that heifers fed the high-fat DDGS had greater concentrations of plasma cholesterol. Other researchers have also reported increased concentrations of plasma cholesterol with increased dietary concentrations of dietary fat (Park et al. 1983; Talavera et al., 1985; Thomas et al., 1997; Funston, 2004). Previous research has reported an inverse relationship between dietary CP and cholesterol suggesting that CP is required for the synthesis of cholesterol or that a protein deficiency hastens cholesterol synthesis (Park et al., 1980). However, this cannot explain the results in the current experiment.

There was a tendency for concentration of serum glucose to decrease with the DDG diet compared to the CON diet. Anderson et al. (2015d) reported similar results. Heifers fed a high-fat DDGS diet had decreased concentrations of serum glucose when compared to the corn and soybean product control diet. However, the forage was not offered ad libitum in this study. This decrease in serum glucose may be attributed to the increased dietary fat in the DDG diet (Park et al., 1980). Concentrations of blood glucose decreased as heifers were fed diets with elevated fat concentrations (Park et al., 1980). It was speculated that the changes in rumen fermentation with increased dietary fat decreased glucogenic nutrient availability and decreased propionate production which is glucogenic (Park et al., 1980). However, the propionate molar percentage was greater for the DDG diet in the current study. There was a tendency for a decrease in the concentration of plasma urea nitrogen in the DDG compared to the CON diet. This may be the result of the intestinal digestion of the RUP in the DDG ration (Kleinschmit et al., 2007). The differences in the concentration of plasma urea nitrogen are inconsistent with Anderson et al. (2015b) who found no differences between the control and high-fat DDGS diets.

Concentration of plasma insulin increased in the DDG compared to the CON diet. Previous research has indicated that concentration of serum insulin increased in heifers fed ad libitum compared to limit-fed (Sejrsen et al., 1983). However, in the current study, heifers were fed grass hay ad libitum and had lower concentrations of plasma insulin compared to heifers from previous experiments in which limit-feeding strategies were utilized (Anderson et al., 2015b; Chapter 3). The differences in the current study may be attributed to the tendency for differences in concentration of serum glucose, but more research is warranted.

Total Tract Nutrient Digestion

Total tract nutrient digestibility is presented in Table 29. There were no differences in digestibility among treatments for any of the nutrients measured. This is inconsistent with findings by Anderson et al. (2015a) who reported an increase in the digestibility of CP and fiber in high-fat DDGS diets. The nutrient digestibility in the current experiment is also less than that reported by other researchers that have fed limit-fed diets containing DDGS (Anderson et al., 2015a; Chapter 2). The ad libitum forage in the current study may explain these differences. Diets that are limit-fed typically result in an increase in energy and nutrient utilization (Zanton and Heinrichs, 2007).

Conclusion

As originally hypothesized, growth performance of the heifers was maintained when feeding a DDG concentrate mix with ad libitum hay. However, feeding a DDG concentrate mix compared to a CON concentrate mix with ad libitum hay did not increase feed efficiency. There were no differences in BW, ADG, or any of the frame growth parameters measured between treatments. A shift in the metabolic profile was demonstrated, but heifers maintained energy status. This demonstrates that producers can limit-feed a DDGS based concentrate mix with ad libitum grass hay to maintain heifer growth, ADG, and metabolic profile compared to a corn and soybean product based concentrate mix..

Table 20. Formulated ingredient and estimated nutrient composition of treatment diets with control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix limit-fed with ad libitum grass hay to growing replacement dairy heifers

	Treatment[1]	
Item	CON	DDG
Ingredient, % DM[2]		
Grass hay	68.5	68.5
DDGS	0.0	30.0
Ground corn	12.0	0.0
Soybean meal	8.12	0.0
Expellers Soybean Meal	6.27	0.0
Soyhulls	3.65	0.0
Vitamin and mineral premix[3]	0.75	0.75
Calcium carbonate	0.38	0.38
Salt	0.38	0.38
Nutrient, % DM[4]		
CP	13.4	13.4
RDP	7.4	7.4
RUP	6.0	6.0
NDF	46.3	49.2
ADF	30.1	31.5
EE	3.0	4.9
ME, Mcal/kg DM	2.35	2.36
NE_G, Mcal/kg DM	0.87	0.88

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Formulated using NRC, 2001.

[3] Contained: 3.19 g/kg of lasalocid, 20.8% Ca, 26.7% NaCl, 1.6% Mg, 0.5% K, 880 ppm Cu, 50 ppm I, 25 ppm Se, 3,880 ppm Zn, 550,000 IU/kg Vitamin A, 110,000 IU/kg Vitamin D_3, and 4,180 IU/kg Vitamin E.

[4] Estimated by NRC, 2001.

Table 21. Nutrient composition of major ingredients used in the control (**CON**) and distillers dried grains with solubles (**DDG**) concentrate mixes of the treatment rations

Item[1,2], % DM	Ground corn	Soybean meal	Expellers soybean meal	Soyhulls
DM, %	84.2	88.3	89.6	89.6
Ash	1.30	6.47	6.50	5.16
OM	98.7	93.5	93.5	94.8
CP	8.17	52.4	47.8	13.4
ADF	2.59	4.78	8.86	46.8
NDF	9.33	8.41	22.75	63.0
EE[3]	3.09	0.85	7.25	1.01
NFC[4]	21.9	68.1	84.3	82.5

[1] % DM, unless otherwise indicated.

[2] Results from analysis of 3-batch composites (n = 1).

[3] EE = ether extract.

[4] %NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001).

Table 22. Nutrient composition of the control (**CON**) and distillers dried grains with solubles (**DDG**) concentrate mixes and forage

	Concentrate Mix				Forage	
	CON		DDG		Grass Hay	
Item[1,2], % DM	Mean	SE	Mean	SE	Mean	SE
DM[3], %	86.7	0.13	88.4	0.31	88.5	0.40
Ash[3]	4.30	0.02	5.15	0.06	9.05	0.18
OM[3]	95.7	0.02	94.9	0.06	91.0	0.18
CP[3]	29.5	0.15	30.6	0.08	6.68	0.12
ADF[3]	10.0	0.18	11.9	0.15	37.3	0.77
NDF[3]	19.2	0.25	34.8	0.25	65.2	0.90
EE[3]	2.97	0.11	10.2	0.09	1.54	0.14
NFC[3,4]	44.0	0.26	19.2	0.28	17.6	0.84
Starch[5]	29.3	0.19	4.27	0.13	0.38	0.040
Ca[5]	0.28	0.010	0.11	0.000	0.35	0.020
P[5]	0.48	0.010	0.94	0.010	0.23	0.005
Mg[5]	0.26	0.005	0.35	0.005	0.20	0.005
K[5]	1.61	0.040	1.20	0.005	2.04	0.070
S[5]	0.27	0.000	0.73	0.005	0.15	0.01
Na[5]	0.01	0.000	0.26	0.010	0.01	0.000
Cl[5]	0.08	0.005	0.20	0.010	0.75	0.040

[1] % DM, unless otherwise indicated.

[2] Does not include mineral mix. Mineral mix was added at feeding.

[3] Results from analysis of monthly composites (n = 6).

[4] %NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001). EE = ether extract.

[5] Results from analysis of 3-mo composites (n = 2).

Table 23. Mean ration composition based on intakes for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatment[1]			*P-value*[2]		
Nutrient, %	CON	DDG	SEM	Trt	wk	Trt × wk
DM[3]	89.2	89.8	0.10	<0.01	<0.01	0.92
Ash[3]	9.55	9.75	0.091	0.15	<0.01	0.45
OM[3]	102.6	101.7	0.07	<0.01	<0.01	0.97
CP[3]	15.6	15.0	0.40	0.26	<0.01	0.96
NDF[3]	55.6	62.4	0.80	<0.01	<0.01	0.58
ADF[3]	31.5	33.1	0.53	0.04	<0.01	0.88
EE[3,4]	2.21	4.45	0.095	<0.01	<0.01	<0.01
NFC[3]	29.2	19.9	0.40	<0.01	<0.01	<0.01
Starch[5]	10.8	1.63	0.42	<0.01	<0.01	<0.01
Ca[5]	0.60	0.51	0.020	<0.01	<0.01	0.68
P[5]	0.34	0.48	0.008	<0.01	<0.01	<0.01
Mg[5]	0.24	0.27	0.002	<0.01	<0.01	0.05
K[5]	2.15	2.01	0.012	<0.01	<0.01	0.56
S[5]	0.21	0.34	0.005	<0.01	<0.01	<0.01
Na[5]	0.25	0.29	0.008	<0.01	<0.01	0.60
Cl[5]	0.91	0.91	0.013	0.85	<0.01	0.52

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significance of effects for treatment (**Trt**), week (**wk**), and treatment × week (**Trt × wk**).

[3] Results from analysis of monthly composites (n = 6).

[4] EE = ether extract.

[5] Results from analysis of 3-mo composites (n = 2).

Table 24. Mean nutrient intakes amounts for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatment[1]			*P-value*[2]		
Nutrient, kg/d	CON	DDG	SEM	Trt	wk	Trt × wk
DM[3]	6.40	6.62	0.266	0.57	<0.01	0.63
Ash	0.62	0.65	0.028	0.42	<0.01	0.61
OM[3]	6.57	6.74	0.274	0.67	<0.01	0.67
CP[3]	0.97	0.97	0.027	0.94	<0.01	0.65
NDF[3]	3.62	4.15	0.199	0.07	<0.01	0.47
ADF[3]	2.04	2.21	0.114	0.33	<0.01	0.59
EE[3,4]	0.14	0.29	0.007	<0.01	<0.01	<0.01
NFC[3]	1.85	1.33	0.052	<0.01	<0.01	0.49
Starch[5]	0.66	0.10	0.014	<0.01	<0.01	<0.01
Ca[5]	0.039	0.034	0.0016	0.07	<0.01	0.87
P[5]	0.021	0.031	0.0009	<0.01	<0.01	<0.01
Mg[5]	0.016	0.018	0.0006	0.03	<0.01	0.44
K[5]	0.14	0.13	0.006	0.68	<0.01	0.73
S[5]	0.013	0.022	0.0006	<0.01	<0.01	<0.01
Na[5]	0.015	0.018	0.0004	<0.01	<0.01	<0.01
Cl[5]	0.058	0.060	0.0026	0.54	<0.01	0.62

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significance of effects for treatment (**Trt**), week (**wk**), and treatment × week (**Trt × wk**).

[3] Results from analysis of monthly composites (n = 6).

[4] EE = ether extract, petroleum.

[5] Results from analysis of 3-mo composites (n = 2).

Table 25. Dry matter intake (**DMI**), body weight (**BW**), and gain to feed ratios for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatments[1]			*P-value*[2]		
Item	CON	DDG	SEM	Trt	Wk	Trt × Wk
Age, initial	218.7	218.4	1.84	0.87	-	-
Body weight, kg						
Mean	269.3	266.3	9.84	0.83	<0.01	0.57
Initial	229.9	229.6	4.01	0.92		
Final	320.8	317.7	10.11			
ADG, kg/d[3]	0.95 ± 0.087	0.94 ± 0.107		0.94		
ADG, kg/d[4]	0.99	0.96	0.050	0.73	<0.01	0.27
Dry matter intake, kg/d						
Mean	6.40	6.62	0.266	0.57	<0.01	0.63
Final	7.92	8.48	0.357			
Gain:Feed[3]	0.167	0.163	0.0070	0.67	<0.01	0.99
Gain:Feed[4]	0.168	0.156	0.0099	0.39	<0.01	0.24
DMI, % BW						
Mean	2.30	2.37	0.142	0.74	<0.01	0.96
Final	2.45	2.6	0.182			

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significant of effects of treatment (**Trt**), week (**wk**), and treatment × week (**Trt × wk**).

[3] Calculated using regression analysis of BW of the d of the study.

[4] Calculated based on change per two week intervals.

Table 26. Frame size measurements for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatments[1]			*P-value*[2]		
Item	CON	DDGS	SEM	Trt	Wk	Trt × Wk
Withers height, cm						
Mean	119.2	119.2	0.39	0.97	<0.01	0.28
Initial	115.6	113.6	0.77	<0.01		
Final	124.0	123.6	0.48			
Change[3], cm/d	0.101 ± 0.0128	0.095 ± 0.0143		0.76		
Hip height, cm						
Mean	123.3	122.8	0.38	0.37	<0.01	0.68
Initial	119.6	118.3	0.64			
Final	127.7	126.9	0.53			
Change[3], cm/d	0.097 ± 0.012	0.089 ± 0.012		0.63		
Heart girth, cm						
Mean	140.6	139.9	0.40	0.28	<0.01	0.43
Initial	138.0	136.8	0.79	0.09		
Final	151.2	150.4	0.65			
Change[3], cm/d	0.197 ± 0.017	0.189 ± 0.020		0.76		
Paunch girth, cm						
Mean	179.2	178.2	0.90	0.41	<0.01	0.92
Initial	160.3	162.9	1.08	0.01		
Final	194.9	194.0	1.60			
Change[3], cm/d	0.291 ± 0.022	0.286 ± 0.024		0.90		
Body length, cm						
Mean	117.5	117.3	0.92	0.86	<0.01	0.77
Initial	113.3	113.0	0.64	0.60		
Final	124.7	123.3	1.18			
Change[3], cm/d	0.117 ± 0.014	0.116 ± 0.015		0.97		
Hip width, cm						
Mean	36.65	36.19	0.708	0.65	<0.01	0.95
Initial	35.54	35.38	0.287	0.56		
Final	39.85	39.48	0.723			
Change[3], cm/d	0.059 ± 0.006	0.058 ± 0.007		0.95		
BCS[4]						
Mean	3.10	3.11	0.026	0.68	<0.01	0.62
Initial	2.99	3.05	0.022	<0.01		
Final	3.15	3.15	0.038			

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significant of effects of treatment (**Trt**), week (**wk**), and treatment × week (**Trt × wk**).

[3] Calculated using regression analysis of BW of the d of the study.

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

Table 27. Rumen fermentation parameters of heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatment[1]			*P-value*[2]		
Item	CON	DDG	SEM	Trt	wk	Trt × wk
pH	6.86	6.93	0.082	0.57	0.53	0.78
NH_3-N, mg/dL	8.70	9.40	0.697	0.48	0.18	0.99
Acetate, m*M*	54.4	45.4	1.59	<0.01	0.26	1.00
Propionate, m*M*	16.7	16.5	0.60	0.82	0.19	0.83
Isobutyrate, m*M*	0.06	0.11	0.043	0.37	0.84	0.23
Butyrate, m*M*	6.43	6.73	0.288	0.47	0.51	0.24
Isovalerate, m*M*	0.48	0.36	0.028	<0.01	0.03	0.31
Valerate, m*M*	0.43	0.60	0.027	<0.01	0.90	0.59
Total VFA, m*M*	78.5	69.8	2.40	<0.01	0.26	0.91
Acetate, m*M*/100 m*M*	69.3	65.2	0.39	<0.01	1.00	0.65
Propionate, m*M*/100 m*M*	21.2	23.6	0.23	<0.01	0.41	0.30
Isobutyrate, m*M*/100 m*M*	0.09	0.16	0.062	0.46	0.91	0.29
Butyrate, m*M*/100 m*M*	8.23	9.62	0.251	<0.01	0.57	0.06
Isovalerate, m*M*/100 m*M*	0.62	0.54	0.044	0.19	0.01	0.51
Valerate, m*M*/100 m*M*	0.56	0.87	0.031	<0.01	0.23	0.70
Acetate:Propionate	3.28	2.78	0.048	<0.01	0.53	0.42

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significance of effects for treatment (**Trt**), week (**wk**), and treatment × week (**Trt × wk**).

Table 28. Plasma metabolites and metabolic hormone concentrations for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatment[1]			*P value*[2]		
Item	CON	DDG	SEM	Trt	Wk	Trt × Wk
Cholesterol, mg/dL	81.14	102.2	3.88	<0.01	0.20	0.45
Glucose[3], mg/dL	75.24	71.72	1.28	0.07	<0.01	0.81
Insulin, ng/mL	0.55	0.70	0.041	0.01	<0.01	0.15
Plasma urea nitrogen, mg/dL	12.49	11.59	0.31	0.06	0.49	0.64
Triglycerides, mg/dL	19.64	20.95	0.95	0.34	1.00	0.97

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significance of effects for treatment (**Trt**), week (**wk**), and treatment × week (**Trt × wk**).

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

Table 29. Total tract digestibility of nutrients for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatment[1]			*P-value*[2]
Item, %	CON	DDG	SEM	Trt
DM	60.4	57.6	2.78	0.22
OM	62.9	60.1	2.58	0.19
CP	60.5	55.4	5.45	0.20
NDF	56.5	58.2	2.86	0.43
ADF	51.0	52.5	2.38	0.44

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significance of effects for treatment (**Trt**).

SUMMARY AND CONCLUSIONS

This research presented here increased understanding related to our overall objective, to determine the optimal inclusion amount of DDGS in limit-fed dairy heifer diets. Chapter 2 evaluated the effect of increasing the inclusion amount of DDGS on heifer growth, feed efficiency, rumen fermentation, and total tract digestibility of nutrients. In Chapter 3, the metabolic profile and onset of puberty of heifers fed increasing amounts of DDGS in replacement of forage was investigated. Chapter 4 further investigated long-term post trial performance of heifers fed increasing amounts of DDGS in replacement of forage. Finally, Chapter 5 evaluated the effect of limit-feeding a control or DDGS based concentrate mix with ad libitum grass hay on the growth performance, rumen fermentation, metabolic profile, and total tract digestibility of nutrients in dairy heifers.

In Chapter 2 it was demonstrated that increasing the inclusion amount of DDGS in replacement of forage in limit-fed dairy heifer rations improved gain: feed, maintained growth performance, and did not increase BCS. Digestibility of DM, OM, and CP were also greater for heifers fed greater inclusion amounts of DDGS. Consistent with other research in which DDGS was fed to dairy heifers, there was no difference in growth performance (Schroer et al., 2014; Anderson et al., 2015a).

The metabolic profile of heifers fed increasing amounts of DDGS in replacement of forage was changed as demonstrated in Chapter 3. Total plasma fatty acids and PUFA were altered by increasing dietary inclusion amount of DDGS. There was also a small change in the concentration of plasma cholesterol. However, heifers maintained energy status without accumulating excess adipose tissue as indicated by leptin. There was an increase in plasma proportion and concentration of linoleic and arachidonic acid with increasing amounts of DDGS. This is of interest because of their role in the synthesis of reproductive hormones, however, no there was no difference demonstrated in age or BW at the onset of puberty, but there was an age by treatment interaction.

This leads to the post trial performance data of the heifers limit-fed diets with increasing amounts of DDGS in replacement of forage that is presented in Chapter 4. Limit-feeding diets with DDGS at up to 50% of dietary DM during the prepubertal period decreased age at first service while maintaining growth performance as demonstrated by minor differences in growth parameters observed just prior to and at the time of parturition. Heifers fed increased amounts of DDGS also maintained lactation performance suggesting that the increased dietary inclusion amount of DDGS did not have a detrimental effect on lactation performance. However, more research on the effect of feeding increasing amounts of DDGS to dairy heifers postpubertal heifers may be warranted to further understand the effect on metabolic profile and reproduction.

Finally, Chapter 5 demonstrates that heifers can be limit-fed a DDGS based concentrate mix compared to a control concentrate mix with ad libitum grass hay and maintain growth performance, feed efficiency, rumen fermentation, and total tract digestibility of nutrients. There were no differences in growth performance suggesting that DDGS is a suitable alternative feedstuff for this feeding strategy.

Overall, the results from this research demonstrate that DDGS can be used to replace corn and soybean products or forage in limit-fed dairy heifer rations at inclusion amounts that are greater than originally hypothesized and can maintain heifer growth, metabolic profile, and first lactation performance.

APPENDIX

Table 1. Ingredient composition of treatment diets with increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay limit-fed to growing replacement Holstein dairy heifers

	Treatment[2]		
Item[1]	30DG	40DG	50DG
Ingredient, % DM			
Grass hay	30.0	40.0	50.0
DDGS	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
Nutrient, % DM			
DM, % of diet	87.8	87.5	87.5
CP	16.5	19.4	21.3
RDP	10.3	11.7	12.7
RUP	6.2	6.5	8.6
NDF	52.8	48.3	45.2
ADF	33.8	30.7	28.6
EE	3.5	4.0	4.5
S	0.38	0.45	0.51
ME, Mcal/kg DM	2.24	2.36	2.48
NE_G, Mcal/kg DM	0.79	0.88	0.97

[1] Formulated using NRC, 2001.

[2] 30% dietary inclusion rate of DDGS (**30DG**), 40% dietary inclusion rate of DDGS (**40DG**), 50% dietary inclusion rate of DDGS (**50DG**).

[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 ppm Mn, 1,250 ppm Cu, 70 ppm Co, 70 ppm I, 53 ppm Se, 5,500 ppm Zn, 325 ppm Fe, 704,000 IU/kg Vitamin A, 140,800 IU/kg Vitamin D_3, and 5,280 IU/kg Vitamin E.

Table 2. Nutrient composition of the grass hay and distillers dried grains with solubles (**DDGS**) used in the treatment diets limit-fed to growing Holstein dairy heifers

	Grass hay		DDGS	
Item[1], % DM	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
Mg[4]	0.16	0.009	0.34	0.007
K[4]	1.99	0.292	1.11	0.030
S[4]	0.15	0.009	0.73	0.007
Mn[4], mg/kg	51.0	2.887	18.0	0.000
Zn[4], mg/kg	32.7	2.848	70.7	1.667
Cu[4], mg/kg	26.7	1.453	12.7	0.333
Fe[4], mg/kg	134.0	8.505	118.7	1.667
Na[4]	0.02	0.007	0.04	0.000
Cl[4]	0.59	0.117	0.22	0.009
Mb[4], mg/kg	2.95	0.683	1.57	0.026

[1] % DM, unless otherwise indicated.

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

[3] %NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001). EE = ether extract.

[4] Results from analysis of 4- or 5-mo composites (n = 3).

Table 3. Nutrient composition of treatment diets with increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay limit-fed to growing Holstein dairy heifers

	Treatment[2]					
	30DG		40DG		50DG	
Item[1], % DM	Mean	SE	Mean	SE	Mean	SE
DM[3], %	86.7	0.288	86.7	0.287	86.8	0.289
OM[3]	91.1	0.226	91.5	0.194	91.9	0.162
Ash[3]	8.83	0.226	8.42	0.194	8.02	0.162
CP[3]	16.8	0.320	19.2	0.291	21.5	0.264
ADF[3]	28.9	0.412	26.1	0.391	23.3	0.373
NDF[3]	54.4	0.469	50.8	0.430	47.1	0.398
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	0.629	17.8	0.548	18.9	0.473
Forage NDF[3]	45.5	0.424	38.8	0.362	32.2	0.300
Nonforage NDF[3]	8.95	0.114	11.9	0.153	14.9	0.191
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
Mn[5], mg/kg	71.1	1.977	67.8	1.689	65.5	1.400
Zn[5], mg/kg	84.8	2.280	88.6	2.126	92.4	1.987
Cu[5], mg/kg	31.4	1.088	30.0	0.974	28.6	0.860
Fe[5], mg/kg	140.3	5.759	138.8	4.902	137.3	4.062
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
Mb[5], mg/kg	2.49	0.470	2.35	0.402	2.21	0.335
ME,[6] Mcal/kg of DM	2.27	-	2.39	-	2.51	-
NE_G,[6] Mcal/kg of DM	0.81	-	0.90	-	0.99	-

[1] % DM, unless otherwise indicated.

[2] 30% dietary inclusion rate of DDGS (**30DG**), 40% dietary inclusion rate of DDGS (**40DG**), 50% dietary inclusion rate of DDGS (**50DG**).

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

[4] % NFC =100 - (% Ash + % CP + % NDF + % EE) (NRC, 2001). EE = ether extract.

[5] Results from analysis of 4- or 5-mo 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 limit-fed increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) 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]									
Average	6.49	6.21	5.84	0.169	0.03	<0.01	0.97	<0.01	0.84
Week 2	5.16	5.01	4.64	0.178					
Week 4	5.60	5.35	5.01	0.178					
Week 6	6.00	5.71	5.37	0.178					
Week 8	6.30	6.00	5.60	0.178					
Week 10	6.72	6.43	6.09	0.178					
Week 12	7.05	6.75	6.35	0.178					
Week 14	7.31	7.02	6.62	0.178					
Week 16	7.75	7.37	7.05	0.178					
OM[3]									
Average	5.91	5.68	5.37	0.155	0.06	<0.01	0.98	0.02	0.83
Week 2	4.70	4.59	4.26	0.163					
Week 4	5.10	4.90	4.60	0.163					
Week 6	5.46	5.22	4.94	0.163					
Week 8	5.74	5.49	5.15	0.163					
Week 10	6.12	5.88	5.60	0.163					
Week 12	6.42	6.18	5.84	0.163					
Week 14	6.66	6.42	6.08	0.163					
Week 16	7.06	6.75	6.48	0.163					
CP[3]									
Average	1.09	1.19	1.26	0.033	<0.01	<0.01	0.04	<0.01	0.69
Week 2	0.87	0.96	1.00	0.035					
Week 4	0.94	1.03	1.08	0.035					
Week 6	1.01	1.09	1.16	0.035					
Week 8	1.06	1.15	1.21	0.035					
Week 10	1.13	1.23	1.31	0.035					
Week 12	1.18	1.29	1.37	0.035					
Week 14	1.23	1.34	1.42	0.035					
Week 16	1.30	1.41	1.52	0.035					
NDF[3]									
Average	3.53	3.15	2.75	0.085	<0.01	<0.01	<0.01	<0.01	0.93
Week 2	2.81	2.55	2.19	0.090					
Week 4	3.05	2.72	2.36	0.090					
Week 6	3.26	2.90	2.53	0.090					
Week 8	3.43	3.05	2.64	0.090					
Week 10	3.66	3.26	2.87	0.090					
Week 12	3.83	3.43	2.99	0.090					
Week 14	3.98	3.56	3.12	0.090					
Week 16	4.22	3.74	3.32	0.090					
ForageNDF[3]									
Average	2.95	2.41	1.88	0.065	<0.01	<0.01	<0.01	<0.01	0.95
Week 2	2.34	1.95	1.49	0.069					
Week 4	2.55	2.08	1.61	0.069					
Week 6	2.73	2.22	1.73	0.069					
Week 8	2.87	2.33	1.80	0.069					
Week 10	3.06	2.50	1.96	0.069					
Week 12	3.20	2.62	2.05	0.069					
Week 14	3.32	2.72	2.13	0.069					
Week 16	3.52	2.86	2.27	0.069					
Nonforage									

NDF[3]									
Average	0.58	0.74	0.87	0.021	<0.01	<0.01	0.11	<0.01	0.55
Week 2	0.46	0.60	0.69	0.022					
Week 4	0.50	0.64	0.75	0.022					
Week 6	0.54	0.68	0.80	0.022					
Week 8	0.56	0.72	0.84	0.022					
Week 10	0.60	0.77	0.91	0.022					
Week 12	0.63	0.81	0.95	0.022					
Week 14	0.65	0.84	0.99	0.022					
Week 16	0.69	0.88	1.05	0.022					
EE (diethyl)[3]									
Average	0.33	0.39	0.43	0.011	<0.01	<0.01	<0.01	<0.01	0.64
Week 2	0.27	0.31	0.34	0.011					
Week 4	0.29	0.34	0.37	0.011					
Week 6	0.31	0.36	0.40	0.011					
Week 8	0.33	0.38	0.41	0.011					
Week 10	0.35	0.40	0.45	0.011					
Week 12	0.36	0.42	0.47	0.011					
Week 14	0.38	0.44	0.49	0.011					
Week 16	0.40	0.46	0.52	0.011					
EE (petroleum)[3]									
Average	0.20	0.23	0.26	0.006	<0.01	<0.01	<0.01	<0.01	0.61
Week 2	0.16	0.19	0.20	0.007					
Week 4	0.17	0.20	0.22	0.007					
Week 6	0.18	0.21	0.24	0.007					
Week 8	0.19	0.22	0.25	0.007					
Week 10	0.21	0.24	0.27	0.007					
Week 12	0.22	0.25	0.28	0.007					
Week 14	0.22	0.26	0.29	0.007					
Week 16	0.24	0.28	0.31	0.007					
Starch[4]									
Average	0.15	0.18	0.20	0.005	<0.01	<0.01	<0.01	<0.01	0.68
Week 2	0.12	0.14	0.16	0.005					
Week 4	0.13	0.15	0.17	0.005					
Week 6	0.14	0.17	0.18	0.005					
Week 8	0.15	0.17	0.19	0.005					
Week 10	0.16	0.19	0.21	0.005					
Week 12	0.17	0.20	0.22	0.005					
Week 14	0.17	0.20	0.23	0.005					
Week 16	0.18	0.21	0.24	0.005					
Sulfur[4]									
Average	0.021	0.024	0.026	0.0007	<0.01	<0.01	<0.01	<0.01	0.66
Week 2	0.017	0.019	0.020	0.0007					
Week 4	0.018	0.020	0.022	0.0007					
Week 6	0.019	0.022	0.024	0.0007					
Week 8	0.020	0.023	0.025	0.0007					
Week 10	0.022	0.024	0.027	0.0007					
Week 12	0.023	0.026	0.028	0.0007					
Week 14	0.023	0.027	0.029	0.0007					
Week 16	0.025	0.028	0.031	0.0007					
ME, Mcal/d									
Average	14.7	14.8	14.7	0.405	0.96	<0.01	1.00	0.91	0.78
Week 2	11.7	11.9	11.6	0.428					
Week 4	12.7	12.8	12.6	0.428					
Week 6	13.6	13.6	13.5	0.428					

Week 8	14.3	14.3	14.1	0.428					
Week 10	15.3	15.4	15.3	0.428					
Week 12	16.0	16.1	15.9	0.428					
Week 14	16.6	16.8	16.6	0.428					
Week 16	17.6	17.6	17.7	0.428					
NE$_G$, Mcal/d									
Average	5.25	5.59	5.78	0.154	0.06	<0.01	0.37	0.02	0.72
Week 2	4.18	4.51	4.59	0.162					
Week 4	4.54	4.82	4.96	0.162					
Week 6	4.86	5.14	5.32	0.162					
Week 8	5.11	5.40	5.55	0.162					
Week 10	5.44	5.79	6.03	0.162					
Week 12	5.71	6.07	6.29	0.162					
Week 14	5.92	6.31	6.55	0.162					
Week 16	6.28	6.64	6.98	0.162					

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[1] 30% dietary inclusion rate of DDGS (**30DG**), 40% dietary inclusion rate of DDGS (**40DG**), 50% dietary inclusion rate of DDGS (**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). EE = ether extract.

[4] Results from analysis of 4- or 5-mo composites (n = 3).

Table 5. Dry matter intake, body weight, and gain:feed ratios for Holstein heifers limit-fed increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay

	Treatment[1]				*P-value*[2]				
Item	30	40	50	SEM	Trt	Wk	Trt × Wk	L	Q
Age, initial	198.1	200.3	199.2	1.93	0.49				
BW, kg									
Average	264.0	266.2	266.4	7.15	0.97	<0.01	0.72	0.82	0.91
Initial	206.6	205.1	206.1	1.95	0.85				
Week 2	220.5	219.6	218.6	7.35					
Week 4	233.1	233.7	231.6	7.35					
Week 6	246.2	247.5	246.2	7.37					
Week 8	257.1	259.6	260.1	7.35					
Week 10	270.3	272.9	274.1	7.35					
Week 12	280.4	283.9	285.5	7.35					
Week 14	297.0	299.6	302.2	7.35					
Week 16	307.6	312.5	313.0	7.35					
ADG, Kg/d[3]	0.89 ±0.071	0.94 ±0.083	0.97 ±0.083		0.44				
ADG, kg/d[4]									
Average	0.91	0.96	0.95	0.043	0.67	<0.01	0.99	0.47	0.60
Week 2	0.99	1.04	0.89	0.112					
Week 4	0.90	1.01	0.92	0.112					
Week 6	0.94	0.98	1.04	0.112					
Week 8	0.84	0.86	0.99	0.112					
Week 10	0.94	0.95	1.01	0.112					
Week 12	0.72	0.79	0.81	0.112					
Week 14	1.19	1.11	1.19	0.112					
Week 16	0.76	0.93	0.77	0.112					
DMI, kg									
Average	6.49	6.21	5.84	0.169	0.03	<0.01	0.97	<0.01	0.84
Week 2	5.16	5.01	4.64	0.178					
Week 4	5.60	5.35	5.01	0.178					
Week 6	6.00	5.71	5.37	0.178					
Week 8	6.30	6.00	5.60	0.178					
Week 10	6.72	6.43	6.09	0.178					
Week 12	7.05	6.75	6.35	0.178					
Week 14	7.31	7.02	6.62	0.178					
Week 16	7.75	7.37	7.05	0.178					
Gain:Feed[3]									
Average	0.141	0.156	0.172	0.0051	<0.01	<0.01	0.09	<0.01	0.93
Week 2	0.175	0.191	0.215	0.0056					
Week 4	0.161	0.178	0.196	0.0056					
Week 6	0.150	0.167	0.183	0.0056					
Week 8	0.143	0.159	0.180	0.0056					
Week 10	0.134	0.148	0.162	0.0056					
Week 12	0.128	0.141	0.155	0.0056					
Week 14	0.123	0.136	0.149	0.0056					
Week 16	0.116	0.129	0.139	0.0056					
Gain:Feed[4]									
Average	0.144	0.159	0.165	0.0063	0.06	<0.01	0.99	0.02	0.56
Week 2	0.191	0.207	0.194	0.0203					
Week 4	0.163	0.189	0.183	0.0203					
Week 6	0.154	0.173	0.196	0.0210					
Week 8	0.133	0.147	0.155	0.0210					

Week 10	0.141	0.148	0.170	0.0203					
Week 12	0.103	0.120	0.130	0.0203					
Week 14	0.167	0.162	0.184	0.0203					
Week 16	0.099	0.127	0.108	0.0203					
DMI, % BW									
Average	2.45	2.33	2.19	0.012	<0.01	<0.01	0.79	<0.01	0.59
Week 2	2.34	1.28	2.12	0.025					
Week 4	2.40	2.29	2.17	0.025					
Week 6	2.45	2.31	2.18	0.025					
Week 8	2.46	2.31	2.15	0.025					
Week 10	2.49	2.36	2.22	0.025					
Week 12	2.51	2.38	2.23	0.025					
Week 14	2.46	2.34	2.19	0.025					
Week 16	2.52	2.36	2.26	0.025					

[1] 30% dietary inclusion rate of DDGS (**30DG**), 40% dietary inclusion rate of DDGS (**40DG**), 50% dietary inclusion rate of DDGS (**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 day of the study.

[4] Calculated based on change per 2-wk intervals.

Table 6. Frame size measurements for Holstein heifers limit-fed treatment diets with increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay

	Treatment[1]				*P-value*[2]				
Item	30	40	50	SEM	Trt	Wk	Trt × Wk	L	Q
Withers height, cm									
Average	121.0	121.7	121.6	0.39	0.41	<.0.01	0.88	0.28	0.44
Initial	113.5	113.1	114.5	0.32	<0.01				
Week 2	114.9	115.3	115.4	0.49					
Week 4	117.0	117.7	117.8	0.49					
Week 6	118.6	119.2	119.5	0.50					
Week 8	120.7	121.3	120.9	0.49					
Week 10	122.0	122.8	122.7	0.49					
Week 12	123.7	124.2	124.2	0.49					
Week 14	125.3	125.7	125.3	0.49					
Week 16	125.7	127.1	127.1	0.49					
Change/d[3]	0.114 ±0.009	0.118 ±0.009	0.115 ±0.011	-	0.93				
Hip height, cm									
Average	124.8	124.7	124.8	0.52	0.97	<0.01	0.93	0.99	0.80
Initial	115.3	116.2	117.3	0.51	<0.01				
Week 2	119.1	118.8	119.1	0.57					
Week 4	120.5	120.6	121.0	0.57					
Week 6	122.3	122.3	122.3	0.57					
Week 8	124.1	123.9	124.1	0.57					
Week 10	125.7	125.7	126.0	0.57					
Week 12	127.9	127.4	127.7	0.57					
Week 14	129.0	128.7	128.5	0.57					
Week 16	130.0	130.1	130.2	0.57					
Change/d[3]	0.117 ±0.009	0.116 ±0.009	0.113 ±0.011	-	0.78				
Heart girth, cm									
Average	140.9	140.6	141.0	0.47	0.85	<0.01	0.81	0.94	0.57
Initial	130.9	131.2	130.7	0.79	0.76				
Week 2	132.1	132.3	132.0	0.60					
Week 4	135.3	134.5	134.6	0.60					
Week 6	137.4	137.2	137.4	0.61					
Week 8	139.6	139.5	139.5	0.60					
Week 10	142.1	141.2	142.3	0.60					
Week 12	145.3	144.4	145.0	0.60					
Week 14	146.4	146.6	147.2	0.60					
Week 16	149.1	148.9	149.6	0.60					
Change/d[3]	0.171 ±0.014	0.170 ±0.018	0.181 ±0.015	-	0.65				
Paunch girth, cm									
Average	172.6	173.8	172.4	1.33	0.73	<0.01	0.97	0.90	0.44
Initial	163.7	162.0	162.1	1.02	0.16				
Week 2	162.5	162.7	161.8	1.65					
Week 4	166.7	166.9	165.2	1.65					
Week 6	170.1	171.6	169.0	1.67					
Week 8	172.4	172.8	170.9	1.65					
Week 10	174.2	174.7	174.3	1.65					

Week 12	175.6	177.1	176.4	1.65					
Week 14	179.6	181.7	180.8	1.65					
Week 16	180.0	182.8	180.9	1.65					
Change/d[3]	0.173 ±0.021	0.199 ±0.025	0.201 ±0.019	-	0.37				
Body length, cm									
Average	112.5	112.9	113.1	0.80	0.84	<0.01	0.96	0.56	0.95
Initial	101.0	101.6	101.5	0.44	0.30				
Week 2	107.0	106.6	106.8	0.93					
Week 4	108.7	109.1	109.1	0.93					
Week 6	109.4	109.4	110.2	0.94					
Week 8	111.5	112.7	112.5	0.93					
Week 10	113.5	114.2	114.6	0.93					
Week 12	114.9	115.1	115.4	0.93					
Week 14	116.8	116.9	117.8	0.93					
Week 16	118.0	119.0	118.7	0.93					
Change/d[3]	0.116 ±0.009	0.123 ±0.011	0.123 ±0.010	-	0.63				
Hip width, cm									
Average	35.63	35.82	35.76	0.452	0.95	<0.01	0.79	0.83	0.82
Initial	32.19	32.11	32.43	0.153	0.30				
Week 2	32.95	32.91	32.83	0.476					
Week 4	33.88	33.74	33.74	0.476					
Week 6	34.35	34.59	34.47	0.476					
Week 8	35.27	35.63	35.60	0.476					
Week 10	36.04	36.06	36.24	0.476					
Week 12	36.60	37.13	36.82	0.476					
Week 14	37.73	37.99	37.99	0.476					
Week 16	38.18	38.50	38.42	0.476					
Change/d[3]	0.054 ±0.005	0.058 ±0.006	0.058 ±0.005	-	0.58				
BCS[4]									
Average	3.11	3.12	3.07	0.028	0.34	0.09	0.14	0.24	0.37
Initial	3.17	3.19	3.15	0.018	0.06				
Week 2	3.14	3.08	3.05	0.034					
Week 4	3.10	3.11	3.02	0.034					
Week 6	3.14	3.11	3.08	0.035					
Week 8	3.15	3.13	3.07	0.034					
Week 10	3.09	3.12	3.05	0.034					
Week 12	3.09	3.16	3.08	0.034					
Week 14	3.10	3.14	3.11	0.034					
Week 16	3.08	3.11	3.08	0.035					

[1] 30% dietary inclusion rate of DDGS (**30DG**), 40% dietary inclusion rate of DDGS (**40DG**), 50% dietary inclusion rate of DDGS (**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 days of the study.

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

Table 7. Rumen fermentation parameters of Holstein heifers limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay

	Treatment[1]				*P-value*[2]				
Item	30DG	40DG	50DG	SEM	Trt	Wk	Trt × Wk	L	Q
pH									
Average	6.67	6.54	6.52	0.087	0.42	0.10	0.46	0.23	0.60
Week 12	6.67	6.46	6.49	0.096					
Week 16	6.68	6.62	6.56	0.098					
NH_3-N, mg/dL									
Average	15.4	17.1	19.3	1.03	0.03	0.52	0.25	<0.01	0.84
Week 12	14.7	17.5	18.9	1.13					
Week 16	16.0	16.6	19.7	1.15					
Acetate, m*M*									
Average	43.4	41.9	41.7	1.38	0.63	0.29	0.07	0.38	0.70
Week 12	42.2	43.2	39.7	1.71					
Week 16	44.6	40.6	43.7	1.76					
Propionate, m*M*									
Average	18.1	19.9	22.6	1.03	0.01	<0.01	0.15	<0.01	0.73
Week 12	16.7	19.9	21.5	1.15					
Week 16	19.5	19.9	23.7	1.18					
Isobutyrate, m*M*									
Average	0.87	0.95	0.95	0.037	0.23	0.37	0.03	0.15	0.37
Week 12	0.81	1.00	0.91	0.046					
Week 16	0.93	0.90	0.98	0.048					
Butyrate, m*M*									
Average	8.88	8.58	7.26	0.420	0.02	0.22	0.22	<0.01	0.32
Week 12	8.76	8.68	6.73	0.488					
Week 16	9.00	8.49	7.78	0.500					
Isovalerate, m*M*									
Average	0.48	0.58	0.50	0.029	0.06	0.20	0.53	0.72	0.02
Week 12	0.48	0.61	0.53	0.039					
Week 16	0.49	0.54	0.47	0.040					
Valerate, m*M*									
Average	1.33	1.30	1.24	0.054	0.53	0.07	0.05	0.27	0.82
Week 12	1.26	1.34	1.12	0.071					
Week 16	1.39	1.26	1.36	0.073					
Total VFA, m*M*									
Average	73.1	73.2	74.2	2.43	0.93	0.09	0.08	0.73	0.88
Week 12	70.2	74.7	70.5	2.95					
Week 16	75.9	71.7	78.0	3.04					
Acetate, m*M*/100 m*M*									
Average	59.4	57.3	56.2	0.553	<0.01	0.03	0.27	<0.01	0.52
Week 12	60.1	57.8	56.1	0.629					
Week 16	58.8	56.9	56.2	0.644					
Propionate, m*M*/100 m*M*									
Average	24.7	26.9	30.4	0.81	<0.01	0.05	0.03	<0.01	0.54
Week 12	23.9	26.4	30.6	0.86					
Week 16	25.5	27.4	30.1	0.87					
Isobutyrate, m*M*/100 m*M*									
Average	1.20	1.31	1.28	0.042	0.19	0.48	0.21	0.18	0.21
Week 12	1.17	1.34	1.30	0.051					
Week 16	1.23	1.27	1.26	0.049					

Butyrate, m*M*/100 m*M*									
Average	12.2	11.9	9.8	0.50	<0.01	0.64	0.31	<0.01	0.19
Week 12	12.4	11.8	9.6	0.54					
Week 16	12.1	11.9	10.1	0.55					
Isovalerate, m*M*/100 m*M*									
Average	0.67	0.80	0.68	0.038	0.05	0.03	0.38	0.80	0.02
Week 12	0.69	0.83	0.77	0.052					
Week 16	0.65	0.76	0.60	0.053					
Valerate, m*M*/100 m*M*									
Average	1.80	1.78	1.67	0.058	0.24	0.14	0.22	0.12	0.50
Week 12	1.76	1.80	1.58	0.073					
Week 16	1.84	1.77	1.77	0.075					
Acetate:Propionate									
Average	2.44	2.18	1.90	0.075	<0.01	<0.01	0.02	<0.01	0.86
Week 12	2.53	2.24	1.89	0.079					
Week 16	2.34	2.13	1.91	0.080					

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

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

Table 8. Mean fatty acid intakes for Holstein heifers limit-fed increasing inclusion amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay

	Treatment[1]				*P-value*[2]				
Fatty acid, g/d	30DG	40DG	50DG	SEM	Trt	wk	Trt × Wk	L	Q
C10:0									
Average	5.94	5.44	4.90	0.148	<0.01	<0.01	0.17	<0.01	0.89
Week 2	4.72	4.40	3.89	0.156					
Week 4	5.13	4.69	4.20	0.156					
Week 6	5.49	5.01	4.51	0.156					
Week 8	6.15	5.64	5.11	0.156					
Week 12	6.45	5.92	5.33	0.156					
Week 14	6.69	6.15	5.55	0.156					
Week 16	7.09	6.47	5.91	0.156					
C12:0									
Average	4.00	3.64	3.25	0.099	<0.01	<0.01	0.08	<0.01	0.90
Week 2	3.18	2.94	2.58	0.104					
Week 4	3.45	3.14	2.79	0.104					
Week 6	3.70	3.35	2.99	0.104					
Week 8	3.88	3.52	3.12	0.104					
Week 10	4.14	3.77	3.39	0.104					
Week 12	4.34	3.96	3.53	0.104					
Week 14	4.51	4.11	3.68	0.104					
Week 16	4.78	4.32	3.92	0.104					
C12:1									
Average	10.28	8.90	7.50	0.241	<0.01	<0.01	<0.01	<0.01	0.97
Week 2	8.18	7.19	5.95	0.255					
Week 4	8.88	7.68	6.43	0.255					
Week 6	9.51	8.19	6.89	0.255					
Week 8	9.99	8.61	7.19	0.255					
Week 10	10.65	9.22	7.81	0.255					
Week 12	11.17	9.68	8.15	0.255					
Week 14	11.59	10.06	8.49	0.255					
Week 16	12.29	10.56	9.05	0.255					
C14:0									
Average	9.02	10.99	12.56	0.307	<0.01	<0.01	<0.01	<0.01	0.60
Week 2	7.17	8.88	9.98	0.325					

Week 4	7.79	9.48	10.77	0.325					
Week 6	8.34	10.11	11.55	0.325					
Week 8	8.76	10.63	12.05	0.325					
Week 10	9.34	11.38	13.09	0.325					
Week 12	9.80	11.95	13.66	0.325					
Week 14	10.16	12.42	14.23	0.325					
Week 16	10.77	13.05	15.16	0.325					
C16:0									
Average	26.09	30.29	33.54	0.842	<0.01	<0.01	<0.01	<0.01	0.64
Week 2	20.74	24.48	26.63	0.890					
Week 4	22.54	26.13	28.76	0.890					
Week 6	24.12	27.87	30.84	0.890					
Week 8	25.35	29.30	32.16	0.890					
Week 10	27.03	31.39	34.96	0.890					
Week 12	28.34	32.95	36.47	0.890					
Week 14	29.40	34.24	37.98	0.890					
Week 16	31.17	35.99	40.48	0.890					
C16:1									
Average	5.37	4.48	3.61	0.122	<0.01	<0.01	<0.01	<0.01	0.98
Week 2	4.27	3.62	2.86	0.128					
Week 4	4.64	3.87	3.09	0.128					
Week 6	4.96	4.13	3.32	0.128					
Week 8	5.22	4.34	3.46	0.128					
Week 10	5.56	4.65	3.76	0.128					
Week 12	5.83	4.88	3.92	0.128					
Week 14	6.05	5.07	4.08	0.128					
Week 16	6.41	5.33	4.35	0.128					
C18:0									
Average	3.49	4.14	4.65	0.115	<0.01	<0.01	<0.01	<0.01	0.62
Week 2	2.78	3.34	3.69	0.122					
Week 4	3.02	3.57	3.99	0.122					
Week 6	3.23	3.81	4.28	0.122					
Week 8	3.39	4.00	4.46	0.122					
Week 10	3.62	4.29	4.85	0.122					
Week 12	3.79	4.50	5.06	0.122					
Week 14	3.94	4.68	5.26	0.122					
Week 16	4.17	4.92	5.61	0.122					

C18:1, *cis* 11									
Average	28.32	35.59	41.49	1.000	<0.01	<0.01	<0.01	<0.01	0.58
Week 2	22.52	28.76	32.95	1.058					
Week 4	24.47	30.69	35.57	1.058					
Week 6	26.19	32.74	38.15	1.058					
Week 8	27.52	34.42	39.79	1.058					
Week 10	29.35	36.88	43.25	1.058					
Week 12	30.77	38.70	45.12	1.058					
Week 14	31.92	40.23	46.98	1.058					
Week 16	33.84	42.28	50.08	1.058					
C18:1, *trans* 11									
Average	1.24	1.54	1.79	0.043	<0.01	<0.01	<0.01	<0.01	0.59
Week 2	0.98	1.25	1.42	0.046					
Week 4	1.07	1.33	1.53	0.046					
Week 6	1.15	1.42	1.65	0.046					
Week 8	1.21	1.49	1.72	0.046					
Week 10	1.29	1.60	1.86	0.046					
Week 12	1.35	1.68	1.95	0.046					
Week 14	1.40	1.74	2.03	0.046					
Week 16	1.48	1.83	2.16	0.046					
C18:2, *cis* 9, *cis* 12									
Average	80.36	100.77	117.33	2.832	<0.01	<0.01	<0.01	<0.01	0.58
Week 2	63.90	81.43	93.18	2.994					
Week 4	69.43	86.91	100.61	2.994					
Week 6	74.31	92.70	107.91	2.994					
Week 8	78.09	97.47	112.53	2.994					
Week 10	83.26	104.42	122.31	2.994					
Week 12	87.29	109.59	127.61	2.994					
Week 14	90.57	113.91	132.89	2.994					
Week 16	96.02	119.73	141.63	2.994					
C18:3 γ									
Average	1.25	1.43	1.57	0.040	<0.01	<0.01	<0.01	<0.01	0.66
Week 2	0.99	1.16	1.24	0.042					
Week 4	1.08	1.23	1.34	0.042					
Week 6	1.16	1.32	1.44	0.042					
Week 8	1.21	1.38	1.50	0.042					
Week 10	1.29	1.48	1.63	0.042					

Week 12	1.36	1.56	1.70	0.042					
Week 14	1.41	1.62	1.78	0.042					
Week 16	1.49	1.70	1.89	0.042					
C20:0									
Average	23.58	22.69	21.47	0.617	0.06	<0.01	0.98	0.02	0.83
Week 2	18.75	18.33	17.05	0.652					
Week 4	20.37	19.57	18.41	0.652					
Week 6	21.80	20.87	19.75	0.652					
Week 8	22.91	21.94	20.59	0.652					
Week 10	24.43	23.51	22.38	0.652					
Week 12	25.61	24.67	23.35	0.652					
Week 14	26.57	25.64	24.32	0.652					
Week 16	28.17	26.95	25.92	0.652					
C18:3 α									
Average	25.71	23.51	21.11	0.638	<0.01	<0.01	0.13	<0.01	0.90
Week 2	20.44	19.00	16.77	0.674					
Week 4	22.21	20.28	18.10	0.674					
Week 6	23.77	21.63	19.42	0.674					
Week 8	24.98	2.74	20.25	0.674					
Week 10	26.63	24.37	22.01	0.674					
Week 12	27.93	25.57	22.96	0.674					
Week 14	28.97	26.58	23.91	0.674					
Week 16	30.72	27.94	25.48	0.674					
C18:2 *trans*[3]									
Average	1.55	1.38	1.21	0.038	<0.01	<0.01	<0.01	<0.01	0.93
Week 2	1.23	1.12	0.96	0.040					
Week 4	1.34	1.19	1.04	0.040					
Week 6	1.43	1.27	1.11	0.040					
Week 8	1.51	1.34	1.16	0.040					
Week 10	1.61	1.43	1.26	0.040					
Week 12	1.68	1.51	1.32	0.040					
Week 14	1.75	1.57	1.37	0.040					
Week 16	1.85	1.65	1.46	0.040					
C20:4									
Average	0.59	0.58	0.55	0.016	0.30	<0.01	1.00	0.13	0.83
Week 2	0.47	0.47	0.44	0.017					
Week 4	0.51	0.50	0.48	0.017					

Week 6	0.54	0.53	0.51	0.017					
Week 8	0.57	0.56	0.53	0.017					
Week 10	0.61	0.60	0.58	0.017					
Week 12	0.64	0.63	0.60	0.017					
Week 14	0.66	0.65	0.53	0.017					
Week 16	0.70	0.68	0.67	0.017					
Others[4]									
Average	18.28	16.60	14.80	0.451	<0.01	<0.01	0.06	<0.01	0.90
Week 2	14.53	13.42	11.75	0.476					
Week 4	15.79	14.32	12.69	0.476					
Week 6	16.90	15.27	13.61	0.476					
Week 8	17.76	16.06	14.19	0.476					
Week 10	18.94	17.20	15.42	0.476					
Week 12	19.85	18.06	16.09	0.476					
Week 14	20.60	18.77	16.76	0.476					
Week 16	21.84	19.73	17.86	0.476					
Total									
Average	245.06	271.99	291.33	5.084	<0.01	<0.01	0.97	<0.01	0.18
Week 2	194.87	219.78	231.36	7.936					
Week 4	211.72	234.58	249.81	7.936					
Week 6	226.61	250.21	267.94	7.936					
Week 8	238.12	263.07	279.41	7.936					
Week 10	253.91	281.84	303.68	7.936					
Week 12	266.21	295.80	316.83	7.936					
Week 14	276.19	307.44	329.94	7.936					
Week 16	292.81	323.16	351.64	7.936					

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[1] 30% dietary inclusion rate of DDGS (**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 9. Plasma metabolites and metabolic hormone concentrations for Holstein heifers limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay

	Treatment[1]				*P value*[2]				
Item	30DG	40DG	50DG	SEM	Trt	Wk	Trt× Wk	L	Q
Cholesterol, mg/dL									
Average	93.48	89.15	97.13	2.96	0.17	<0.01	0.39	0.39	0.10
Initial	72.09	68.81	74.12	2.89	0.10				
Week 4	90.01	85.29	89.23	3.72					
Week 8	89.46	86.07	92.03	3.72					
Week 12	95.87	94.58	101.63	3.72					
Week 16	98.58	90.67	105.63	3.72					
Glucose[3], mg/dL									
Average	76.26	77.74	77.33	1.67	0.81	0.10	0.88	0.65	0.65
Initial	89.90	89.45	89.64	1.56	0.95				
Week 4	78.32	78.67	78.95	2.13					
Week 8	74.68	77.40	75.00	2.13					
Week 12	74.62	77.51	77.37	2.13					
Week 16	77.43	77.37	77.98	2.13					
IGF-1, ng/mL									
Average	102.7	100.0	109.4	4.27	0.29	<0.01	0.30	0.27	0.25
Initial	120.7	105.6	123.4	5.56	<0.01				
Week 4	88.67	91.86	100.6	5.68					
Week 8	95.94	94.81	107.8	5.68					
Week 12	109.2	105.8	105.4	5.68					
Week 16	117.1	107.5	123.8	5.68					
Insulin, ng/mL									
Average	1.05	1.12	1.15	0.099	0.78	<0.01	0.61	0.50	0.84
Initial	1.57	1.49	1.64	0.091	0.21				
Week 4	0.87	0.95	0.97	0.131					
Week 8	0.99	1.09	1.16	0.131					
Week 12	0.95	1.14	1.21	0.131					

Week 16	1.38	1.30	1.25	0.131					
Leptin, ng/mL									
Average	4.42	4.35	4.59	0.091	0.19	0.14	0.57	0.22	0.18
Initial	4.48	4.39	4.65	0.088	0.11				
Week 4	4.65	4.23	4.57	0.170					
Week 8	4.48	4.56	4.64	0.170					
Week 12	4.51	4.40	4.61	0.170					
Week 16	4.06	4.21	4.52	0.170					
Plasma urea nitrogen, mg/dL									
Average	17.83	17.82	19.90	0.495	<0.01	<0.01	0.90	<0.01	0.09
Initial	16.08	14.87	16.16	0.431	<0.01				
Week 4	16.33	16.80	18.53	0.692					
Week 8	16.79	17.28	19.42	0.692					
Week 12	19.35	18.53	20.84	0.692					
Week 16	18.85	18.65	20.81	0.692					
Triglycerides, mg/dL									
Average	17.82	19.14	18.47	0.643	0.36	0.89	0.54	0.48	0.21
Initial	18.41	17.49	14.84	0.673	<0.01				
Week 4	19.51	19.41	17.22	1.396					
Week 8	17.29	19.44	17.25	1.396					
Week 12	17.50	18.09	19.53	1.396					
Week 16	16.99	19.63	19.89	1.396					

[1] 30% dietary inclusion rate of DDGS (**30DG**); 40% dietary inclusion rate of DDGS (**40DG**); 50% dietary inclusion rate of DDGS (**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 10. Milk production performance based on Dairy Herd Improvement Association (**DHIA**) records for Holstein heifers limit-fed increasing amounts of distillers dried grains with solubles (**DDGS**) in replacement of grass hay

	Treatment[1]				*P-value*[2]				
Item	30DG	40DG	50DG	SEM	Trt	Mo	Trt × Mo	L	Q
Milk yield, kg									
Average	27.4	28.8	29.4	1.85	0.74	<0.01	0.30	0.46	0.84
Month 1	25.8	23.6	24.3	2.25					
Month 2	27.2	31.2	29.7	2.36					
Month 3	29.3	31.7	34.0	2.61					
ECM[3], kg									
Average	19.3	19.8	20.2	1.17	0.84	0.03	0.40	0.56	0.93
Month 1	19.5	16.8	17.7	1.62					
Month 2	18.5	20.8	20.8	1.70					
Month 3	19.8	21.8	22.1	1.93					
Fat, %									
Average	4.54	4.66	4.66	0.29	0.94	<0.01	0.61	0.76	0.85
Month 1	5.29	5.29	5.22	0.41					
Month 2	4.30	4.23	4.86	0.43					
Month 3	4.03	4.46	3.91	0.49					
Fat yield, kg/d									
Average	0.59	0.59	0.60	0.040	0.99	0.96	0.40	0.90	0.93
Month 1	0.66	0.53	0.57	0.066					
Month 2	0.54	0.60	0.64	0.070					
Month 3	0.57	0.65	0.59	0.070					
Protein, %									
Average	2.88	2.92	2.96	0.08	0.80	0.07	0.92	0.51	0.96
Month 1	2.94	3.04	3.01	0.10					
Month 2	2.84	2.82	2.87	0.10					
Month 3	2.86	2.92	2.98	0.10					
Protein yield, kg/d									
Average	0.36	0.38	0.39	0.024	0.65	<0.01	0.24	0.36	0.97
Month 1	0.34	0.32	0.33	0.029					
Month 2	0.35	0.39	0.38	0.030					
Month 3	0.38	0.41	0.46	0.030					
Somatic cells, × 10^3/mL									
Average	451.0	132.6	113.4	84.0	0.01	0.06	0.02	<0.01	0.12
Month 1	357.8	75.2	130.9	147.1					
Month 2	959.7	106.9	134.2	156.3					
Month 3	35.4	215.5	75.1	167.8					

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

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

[3] ECM = [(0.327 × kg of milk) + (12.95 × kg of fat) + (7.2 × kg of protein)] (Orth, 1992).

Table 11. Mean ration composition for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatment[1]			*P-value*[2]		
Nutrient, %	CON	DDG	SEM	Trt	Wk	Trt × Wk
DM[3]						
Mean	89.2	89.8	0.10	<0.01	<0.01	0.92
Week 2	90.7	91.4	0.24			
Week 4	89.7	90.4	0.24			
Week 6	88.7	89.1	0.24			
Week 8	88.9	89.3	0.24			
Week 10	89.1	89.6	0.24			
Week 12	88.9	89.8	0.24			
Week 14	88.7	89.2	0.24			
Week 16	88.8	89.2	0.24			
Ash[3]						
Mean	9.55	9.75	0.091	0.15	<0.01	0.45
Week 2	9.38	9.46	0.119			
Week 4	9.54	9.79	0.119			
Week 6	9.47	9.66	0.119			
Week 8	9.37	9.54	0.119			
Week 10	9.49	9.66	0.119			
Week 12	9.57	9.58	0.119			
Week 14	9.81	10.2	0.119			
Week 16	9.80	10.1	0.119			
OM[3]						
Mean	102.6	101.7	0.07	<0.01	<0.01	0.97
Week 2	101.0	100.0	0.27			
Week 4	102.0	100.8	0.27			
Week 6	103.3	102.6	0.27			
Week 8	103.1	102.4	0.27			
Week 10	102.8	102.0	0.27			
Week 12	102.9	101.7	0.27			
Week 14	102.9	102.0	0.27			
Week 16	102.9	101.9	0.27			
CP[3]						
Mean	15.6	15.0	0.40	0.26	<0.01	0.96
Week 2	17.1	16.4	0.51			
Week 4	16.9	16.2	0.51			
Week 6	14.8	14.7	0.51			
Week 8	14.9	14.1	0.51			
Week 10	14.9	14.2	0.51			
Week 12	15.2	14.7	0.51			
Week 14	15.4	14.9	0.51			
Week 16	15.6	14.7	0.51			
NDF[3]						
Mean	55.6	62.4	0.80	<0.01	<0.01	0.58
Week 2	52.6	60.7	1.06			
Week 4	53.8	61.6	1.06			
Week 6	57.8	63.1	1.06			
Week 8	57.4	63.9	1.06			
Week 10	56.4	62.8	1.06			
Week 12	55.7	61.9	1.06			
Week 14	55.9	62.2	1.06			
Week 16	55.4	62.6	1.06			
ADF[3]						
Mean	31.5	33.1	0.53	0.04	<0.01	0.88

Week 2	30.1	32.1	0.69			
Week 4	30.8	32.7	0.69			
Week 6	32.9	33.6	0.69			
Week 8	32.6	34.2	0.69			
Week 10	31.7	33.5	0.69			
Week 12	31.4	32.8	0.69			
Week 14	31.2	32.7	0.69			
Week 16	31.0	33.0	0.69			
EE[3]						
Mean	2.21	4.45	0.095	<0.01	<0.01	<0.01
Week 2	2.27	4.94	0.129			
Week 4	2.25	4.81	0.129			
Week 6	2.07	4.24	0.129			
Week 8	2.08	4.02	0.129			
Week 10	2.22	4.26	0.129			
Week 12	2.24	4.47	0.129			
Week 14	2.27	4.47	0.129			
Week 16	2.29	4.39	0.129			
NFC[3]						
Mean	29.2	19.9	0.40	<0.01	<0.01	<0.01
Week 2	29.0	17.9	0.56			
Week 4	29.1	18.2	0.56			
Week 6	28.6	20.5	0.56			
Week 8	28.7	20.5	0.56			
Week 10	29.3	20.7	0.56			
Week 12	29.7	20.7	0.56			
Week 14	29.4	20.4	0.56			
Week 16	29.6	20.3	0.56			
Starch[4]						
Mean	10.8	1.63	0.42	<0.01	<0.01	<0.01
Week 2	13.0	1.92	0.53			
Week 4	12.7	1.88	0.53			
Week 6	9.84	1.60	0.53			
Week 8	10.0	1.49	0.53			
Week 10	9.98	1.51	0.53			
Week 12	10.4	1.59	0.53			
Week 14	10.2	1.57	0.53			
Week 16	10.5	1.52	0.53			
Ca[4]						
Mean	0.60	0.51	0.020	<0.01	<0.01	0.68
Week 2	0.57	0.46	0.025			
Week 4	0.61	0.53	0.025			
Week 6	0.59	0.52	0.025			
Week 8	0.57	0.48	0.025			
Week 10	0.59	0.51	0.025			
Week 12	0.62	0.51	0.025			
Week 14	0.63	0.56	0.025			
Week 16	0.64	0.55	0.025			
P[4]						
Mean	0.34	0.48	0.008	<0.01	<0.01	<0.01
Week 2	0.36	0.54	0.010			
Week 4	0.36	0.52	0.010			
Week 6	0.34	0.47	0.010			
Week 8	0.34	0.46	0.010			
Week 10	0.33	0.46	0.010			
Week 12	0.34	0.47	0.010			

Week 14	0.33	0.47	0.010			
Week 16	0.33	0.46	0.010			
Mg[4]						
Mean	0.24	0.27	0.002	<0.01	<0.01	0.05
Week 2	0.23	0.26	0.002			
Week 4	0.24	0.27	0.002			
Week 6	0.24	0.26	0.002			
Week 8	0.24	0.26	0.002			
Week 10	0.24	0.26	0.002			
Week 12	0.24	0.26	0.002			
Week 14	0.25	0.27	0.002			
Week 16	0.25	0.27	0.002			
K[4]						
Mean	2.15	2.01	0.012	<0.01	<0.01	0.56
Week 2	2.13	1.96	0.019			
Week 4	2.14	1.99	0.019			
Week 6	2.19	2.07	0.019			
Week 8	2.19	2.09	0.019			
Week 10	2.16	2.04	0.019			
Week 12	2.15	2.02	0.019			
Week 14	2.11	1.97	0.019			
Week 16	2.10	1.98	0.019			
S[4]						
Mean	0.21	0.34	0.005	<0.01	<0.01	<0.01
Week 2	0.20	0.37	0.007			
Week 4	0.21	0.37	0.007			
Week 6	0.20	0.33	0.007			
Week 8	0.20	0.32	0.007			
Week 10	0.20	0.33	0.007			
Week 12	0.21	0.34	0.007			
Week 14	0.22	0.35	0.007			
Week 16	0.22	0.34	0.007			
Na[4]						
Mean	0.25	0.29	0.008	<0.01	<0.01	0.60
Week 2	0.29	0.32	0.013			
Week 4	0.28	0.34	0.013			
Week 6	0.23	0.28	0.013			
Week 8	0.23	0.25	0.013			
Week 10	0.23	0.26	0.013			
Week 12	0.24	0.27	0.013			
Week 14	0.24	0.29	0.013			
Week 16	0.24	0.28	0.013			
Cl[4]						
Mean	0.91	0.91	0.013	0.85	<0.01	0.52
Week 2	0.92	0.90	0.017			
Week 4	0.93	0.95	0.017			
Week 6	0.93	0.94	0.017			
Week 8	0.92	0.91	0.017			
Week 10	0.90	0.90	0.017			
Week 12	0.91	0.90	0.017			
Week 14	0.87	0.89	0.017			
Week 16	0.87	0.88	0.017			

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significance of effects for treatment (**Trt**), week (**Wk**), and treatment × week (**Trt × Wk**).

[3] Results from analysis of monthly composites (n = 6). EE = ether extract.

[4] Results from analysis of 3-mo composites (n = 2).

Table 12. Mean nutrient intake amounts for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatment[1]			*P-value*[2]		
Nutrient, kg/d	CON	DDG	SEM	Trt	Wk	Trt × Wk
DM[3]						
Mean	6.40	6.62	0.266	0.57	<0.01	0.63
Week 2	4.13	4.00	0.357			
Week 4	4.78	4.74	0.357			
Week 6	6.18	6.18	0.357			
Week 8	6.41	6.94	0.357			
Week 10	6.86	7.48	0.357			
Week 12	7.04	7.31	0.357			
Week 14	7.86	7.82	0.357			
Week 16	7.92	8.48	0.357			
Ash[3]						
Mean	0.62	0.65	0.028	0.42	<0.01	0.61
Week 2	0.39	0.38	0.038			
Week 4	0.46	0.46	0.038			
Week 6	0.58	0.60	0.038			
Week 8	0.60	0.66	0.038			
Week 10	0.65	0.72	0.038			
Week 12	0.68	0.70	0.038			
Week 14	0.78	0.79	0.038			
Week 16	0.78	0.86	0.038			
OM[3]						
Mean	6.57	6.74	0.274	0.67	<0.01	0.67
Week 2	4.17	4.00	0.371			
Week 4	4.88	4.78	0.371			
Week 6	6.38	6.34	0.371			
Week 8	6.61	7.11	0.371			
Week 10	7.05	7.63	0.371			
Week 12	7.25	7.44	0.371			
Week 14	8.09	7.97	0.371			
Week 16	8.15	8.65	0.371			
CP[3]						
Mean	0.97	0.97	0.027	0.94	<0.01	0.65
Week 2	0.69	0.65	0.034			
Week 4	0.78	0.76	0.034			
Week 6	0.91	0.90	0.034			
Week 8	0.95	0.97	0.034			
Week 10	1.01	1.05	0.034			
Week 12	1.06	1.06	0.034			
Week 14	1.16	1.15	0.034			
Week 16	1.20	1.23	0.034			
NDF[3]						
Mean	3.62	4.15	0.199	0.07	<0.01	0.47
Week 2	2.21	2.44	0.264			
Week 4	2.63	2.93	0.264			
Week 6	3.58	3.91	0.264			
Week 8	3.70	4.45	0.264			
Week 10	3.88	4.71	0.264			
Week 12	3.96	4.56	0.264			
Week 14	4.50	4.89	0.264			
Week 16	4.47	5.32	0.264			
ADF[3]						
Mean	2.04	2.21	0.114	0.33	<0.01	0.59

Week 2	1.27	1.29	0.151			
Week 4	1.51	1.56	0.151			
Week 6	2.04	2.08	0.151			
Week 8	2.10	2.39	0.151			
Week 10	2.19	2.52	0.151			
Week 12	2.23	2.42	0.151			
Week 14	2.52	2.57	0.151			
Week 16	2.49	2.81	0.151			
EE[3]						
Mean	0.14	0.29	0.007	<0.01	<0.01	<0.01
Week 2	0.09	0.19	0.009			
Week 4	0.11	0.22	0.009			
Week 6	0.13	0.26	0.009			
Week 8	0.13	0.28	0.009			
Week 10	0.15	0.31	0.009			
Week 12	0.16	0.32	0.009			
Week 14	0.18	0.34	0.009			
Week 16	0.18	0.37	0.009			
NFC[3]						
Mean	1.85	1.33	0.052	<0.01	<0.01	0.49
Week 2	1.18	0.71	0.076			
Week 4	1.36	0.87	0.076			
Week 6	1.76	1.27	0.076			
Week 8	1.83	1.42	0.076			
Week 10	2.00	1.55	0.076			
Week 12	2.07	1.51	0.076			
Week 14	2.26	1.59	0.076			
Week 16	2.30	1.73	0.076			
Starch[4]						
Mean	0.66	0.10	0.014	<0.01	<0.01	<0.01
Week 2	0.52	0.08	0.015			
Week 4	0.58	0.09	0.015			
Week 6	0.60	0.10	0.015			
Week 8	0.63	0.10	0.015			
Week 10	0.68	0.11	0.015			
Week 12	0.71	0.11	0.015			
Week 14	0.74	0.12	0.015			
Week 16	0.78	0.13	0.015			
Ca[4]						
Mean	0.039	0.034	0.0016	0.07	<0.01	0.87
Week 2	0.024	0.019	0.0020			
Week 4	0.029	0.025	0.0020			
Week 6	0.036	0.032	0.0020			
Week 8	0.036	0.033	0.0020			
Week 10	0.040	0.038	0.0020			
Week 12	0.043	0.037	0.0020			
Week 14	0.049	0.044	0.0020			
Week 16	0.050	0.047	0.0020			
P[4]						
Mean	0.021	0.031	0.0009	<0.01	<0.01	<0.01
Week 2	0.015	0.021	0.0011			
Week 4	0.017	0.024	0.0011			
Week 6	0.021	0.029	0.0011			
Week 8	0.022	0.031	0.0011			
Week 10	0.023	0.034	0.0011			
Week 12	0.024	0.034	0.0011			

Week 14	0.025	0.036	0.0011			
Week 16	0.026	0.039	0.0011			
Mg[4]						
Mean	0.016	0.018	0.0006	0.03	<0.01	0.44
Week 2	0.010	0.010	0.0008			
Week 4	0.011	0.013	0.0008			
Week 6	0.015	0.016	0.0008			
Week 8	0.015	0.018	0.0008			
Week 10	0.017	0.020	0.0008			
Week 12	0.017	0.019	0.0008			
Week 14	0.020	0.021	0.0008			
Week 16	0.020	0.023	0.0008			
K[4]						
Mean	0.14	0.13	0.006	0.68	<0.01	0.73
Week 2	0.09	0.08	0.008			
Week 4	0.10	0.09	0.008			
Week 6	0.14	0.13	0.008			
Week 8	0.14	0.15	0.008			
Week 10	0.15	0.15	0.008			
Week 12	0.15	0.15	0.008			
Week 14	0.17	0.15	0.008			
Week 16	0.17	0.17	0.008			
S[4]						
Mean	0.013	0.022	0.0006	<0.01	<0.01	<0.01
Week 2	0.008	0.015	0.0007			
Week 4	0.010	0.017	0.0007			
Week 6	0.012	0.021	0.0007			
Week 8	0.013	0.022	0.0007			
Week 10	0.014	0.024	0.0007			
Week 12	0.014	0.024	0.0007			
Week 14	0.017	0.027	0.0007			
Week 16	0.017	0.029	0.0007			
Na[4]						
Mean	0.015	0.018	0.0004	<0.01	<0.01	<0.01
Week 2	0.012	0.013	0.0005			
Week 4	0.013	0.016	0.0005			
Week 6	0.014	0.017	0.0005			
Week 8	0.014	0.017	0.0005			
Week 10	0.016	0.019	0.0005			
Week 12	0.017	0.020	0.0005			
Week 14	0.018	0.022	0.0005			
Week 16	0.018	0.023	0.0005			
Cl[4]						
Mean	0.058	0.060	0.0026	0.54	<0.01	0.62
Week 2	0.038	0.036	0.0034			
Week 4	0.045	0.045	0.0034			
Week 6	0.057	0.058	0.0034			
Week 8	0.059	0.063	0.0034			
Week 10	0.062	0.068	0.0034			
Week 12	0.064	0.066	0.0034			
Week 14	0.068	0.069	0.0034			
Week 16	0.069	0.075	0.0034			

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[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significance of effects for treatment (**Trt**), week (**Wk**), and treatment × week (**Trt × Wk**).

[3] Results from analysis of monthly composites (n = 6). EE = ether extract.

[4] Results from analysis of 3-mo composites (n = 2).

Table 13. Dry matter intake, body weight (**BW**), and gain to feed ratios for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatments[1]			*P-value*[2]		
Item	CON	DDG	SEM	Trt	Wk	Trt × Wk
Age, initial	218.7	218.4	1.84	0.87		
Body weight, kg						
Mean	269.3	266.3	9.84	0.83	<0.01	0.57
Initial	229.9	229.6	4.01	0.92		
Week 2	224.2	218.5	10.11			
Week 4	234.5	232.7	10.11			
Week 6	249.4	247.8	10.11			
Week 8	264.1	265.0	10.11			
Week 10	273.6	274.8	10.11			
Week 12	286.1	279.2	10.11			
Week 14	301.4	294.8	10.11			
Week 16	320.8	317.7	10.11			
ADG, kg/d[3]	0.95 ± 0.087	0.94 ± 0.107		0.94		
ADG, kg/d[4]						
Mean	0.99	0.96	0.050	0.73	<0.01	0.27
Week 2	0.99	0.62	0.185			
Week 4	0.74	1.01	0.185			
Week 6	1.06	1.08	0.185			
Week 8	1.05	1.23	0.185			
Week 10	0.68	0.71	0.185			
Week 12	0.89	0.31	0.185			
Week 14	1.10	1.12	0.185			
Week 16	1.39	1.64	0.185			
Dry matter intake, kg/d						
Mean	6.40	6.62	0.266	0.57	<0.01	0.63
Week 2	4.13	4.00	0.357			
Week 4	4.78	4.74	0.357			
Week 6	6.18	6.18	0.357			
Week 8	6.41	6.94	0.357			
Week 10	6.86	7.48	0.357			
Week 12	7.04	7.31	0.357			
Week 14	7.86	7.82	0.357			
Week 16	7.92	8.48	0.357			
Gain:Feed[3]						
Mean	0.167	0.163	0.0070	0.67	<0.01	0.99
Week 2	0.250	0.260	0.0138			
Week 4	0.221	0.216	0.0138			
Week 6	0.161	0.162	0.0138			
Week 8	0.157	0.145	0.0138			
Week 10	0.145	0.136	0.0138			
Week 12	0.144	0.141	0.0138			
Week 14	0.131	0.127	0.0138			
Week 16	0.129	0.118	0.0138			
Gain:Feed[4]						
Mean	0.168	0.156	0.0099	0.39	<0.01	0.24
Week 2	0.258	0.151	0.0342			
Week 4	0.164	0.224	0.0342			
Week 6	0.179	0.184	0.0342			
Week 8	0.170	0.185	0.0342			
Week 10	0.106	0.104	0.0342			
Week 12	0.134	0.042	0.0342			

Week 14	0.147	0.152	0.0342			
Week 16	0.185	0.202	0.0342			
DMI, % BW						
Mean	2.30	2.37	0.142	0.74	<0.01	0.96
Week 2	1.76	1.74	0.182			
Week 4	1.95	1.93	0.182			
Week 6	2.41	2.40	0.182			
Week 8	2.35	2.52	0.182			
Week 10	2.46	2.62	0.182			
Week 12	2.40	2.51	0.182			
Week 14	2.61	2.61	0.182			
Week 16	2.45	2.61	0.182			

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significant of effects of treatment (**Trt**), week (**wk**), and treatment × week (**Trt × wk**).

[3] Calculated using regression analysis of BW of the d of the study.

[4] Calculated based on change per two week intervals.

Table 14. Frame size measurements for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatments[1]			*P-value*[2]		
Item	CON	DDGS	SEM	Trt	Wk	Trt × Wk
Withers height, cm						
Mean	119.2	119.2	0.39	0.97	<0.01	0.28
Initial	115.6	113.6	0.77	<0.01		
Week 2	113.6	114.4	0.48			
Week 4	115.7	116.0	0.48			
Week 6	117.6	117.8	0.48			
Week 8	118.8	119.0	0.48			
Week 10	120.4	120.3	0.48			
Week 12	121.3	120.5	0.48			
Week 14	122.4	122.3	0.48			
Week 16	124.0	123.6	0.48			
Change[3], cm/d	0.101 ± 0.0128	0.095 ± 0.0143		0.76		
Hip height, cm						
Mean	123.3	122.8	0.38	0.37	<0.01	0.68
Initial	119.6	118.3	0.64			
Week 2	118.5	118.8	0.53			
Week 4	119.7	119.6	0.53			
Week 6	121.8	121.3	0.53			
Week 8	123.1	122.5	0.53			
Week 10	124.3	123.8	0.53			
Week 12	124.7	124.0	0.53			
Week 14	127.0	125.9	0.53			
Week 16	127.7	126.9	0.53			
Change[3], cm/d	0.097 ± 0.012	0.089 ± 0.012		0.63		
Heart girth, cm						
Mean	140.6	139.9	0.40	0.28	<0.01	0.43
Initial	138.0	136.8	0.79	0.09		
Week 2	131.2	130.6	0.65			
Week 4	134.6	134.4	0.65			
Week 6	135.2	135.8	0.65			
Week 8	140.1	139.6	0.65			
Week 10	140.9	140.4	0.65			
Week 12	144.8	144.0	0.65			
Week 14	146.6	144.4	0.65			
Week 16	151.2	150.4	0.65			
Change[3], cm/d	0.197 ± 0.017	0.189 ± 0.020		0.76		
Paunch girth, cm						
Mean	179.2	178.2	0.90	0.41	<0.01	0.92
Initial	160.3	162.9	1.08	0.01		
Week 2	162.7	160.9	1.60			
Week 4	171.1	169.8	1.60			
Week 6	174.2	173.1	1.60			
Week 8	179.6	180.7	1.60			
Week 10	179.9	179.7	1.60			
Week 12	184.1	182.9	1.60			
Week 14	187.3	184.2	1.60			
Week 16	194.9	194.0	1.60			
Change[3], cm/d	0.291 ± 0.022	0.286 ± 0.024		0.90		
Body length, cm						
Mean	117.5	117.3	0.92	0.86	<0.01	0.77
Initial	113.3	113.0	0.64	0.60		

Week 2	112.3	111.8	1.18			
Week 4	113.4	113.5	1.18			
Week 6	114.5	114.4	1.18			
Week 8	117.5	117.2	1.18			
Week 10	116.6	115.7	1.18			
Week 12	120.3	120.2	1.18			
Week 14	120.5	122.0	1.18			
Week 16	124.7	123.3	1.18			
Change[3], cm/d	0.117 ± 0.014	0.116 ± 0.015		0.97		
Hip width, cm						
Mean	36.65	36.19	0.708	0.65	<0.01	0.95
Initial	35.54	35.38	0.287	0.56		
Week 2	33.99	33.58	0.723			
Week 4	34.54	33.90	0.723			
Week 6	35.47	35.14	0.723			
Week 8	36.18	35.90	0.723			
Week 10	37.04	36.55	0.723			
Week 12	37.69	37.11	0.723			
Week 14	38.42	37.89	0.723			
Week 16	39.85	39.48	0.723			
Change[3], cm/d	0.059 ± 0.006	0.058 ± 0.007		0.95		
BCS[4]						
Mean	3.10	3.11	0.026	0.68	<0.01	0.62
Initial	2.99	3.05	0.022	<0.01		
Week 2	3.07	3.06	0.038			
Week 4	3.06	3.03	0.038			
Week 6	3.09	3.11	0.038			
Week 8	3.10	3.13	0.038			
Week 10	3.07	3.12	0.038			
Week 12	3.11	3.18	0.038			
Week 14	3.14	3.13	0.038			
Week 16	3.15	3.15	0.038			
Change[3], cm/d	0.0008 ± 0.0004	0.0013 ± 0.0005		0.47		

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[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significant of effects of treatment (**Trt**), week (**wk**), and treatment × week (**Trt × wk**).

[3] Calculated using regression analysis of BW of the d of the study.

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

Table 15. Rumen fermentation parameters of heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDG**) concentrate mix with ad libitum grass hay

	Treatment[1]			*P-value*[2]		
Item	CON	DDG	SEM	Trt	Wk	Trt × Wk
pH						
Mean	6.86	6.93	0.082	0.57	0.53	0.78
Week 12	6.82	6.91	0.106			
Week 16	6.91	9.95	0.106			
NH_3-N, mg/dL						
Mean	8.70	9.40	0.697	0.48	0.18	0.99
Week 12	8.32	9.01	0.803			
Week 16	9.08	9.79	0.803			
Acetate, m*M*						
Mean	54.4	45.4	1.59	<0.01	0.26	1.00
Week 12	55.4	46.5	2.06			
Week 16	53.4	44.4	2.06			
Propionate, m*M*						
Mean	16.7	16.5	0.60	0.82	0.19	0.83
Week 12	17.2	16.9	0.76			
Week 16	16.2	16.1	0.76			
Isobutyrate, m*M*						
Mean	0.06	0.11	0.043	0.37	0.84	0.23
Week 12	0.03	0.15	0.057			
Week 16	0.09	0.08	0.057			
Butyrate, m*M*						
Mean	6.43	6.73	0.288	0.47	0.51	0.24
Week 12	6.36	6.99	0.351			
Week 16	6.50	6.47	0.351			
Isovalerate, m*M*						
Mean	0.48	0.36	0.028	<0.01	0.03	0.31
Week 12	0.43	0.35	0.033			
Week 16	0.52	0.38	0.033			
Valerate, m*M*						
Mean	0.43	0.60	0.027	<0.01	0.90	0.59
Week 12	0.43	0.61	0.033			
Week 16	0.44	0.60	0.033			
Total VFA, m*M*						
Mean	78.5	69.8	2.40	<0.01	0.26	0.91
Week 12	79.9	71.5	3.08			
Week 16	77.1	68.0	3.08			
Acetate, m*M*/100 m*M*						
Mean	69.3	65.2	0.39	<0.01	1.00	0.65
Week 12	69.4	65.1	0.50			
Week 16	69.2	65.3	0.50			
Propionate, m*M*/100 m*M*						
Mean	21.2	23.6	0.23	<0.01	0.41	0.30
Week 12	21.5	23.5	0.31			
Week 16	20.9	23.6	0.31			
Isobutyrate, m*M*/100 m*M*						
Mean	0.09	0.16	0.062	0.46	0.91	0.29
Week 12	0.05	0.21	0.088			
Week 16	0.13	0.11	0.088			
Butyrate, m*M*/100 m*M*						

Mean	8.23	9.62	0.251	<0.01	0.57	0.06
Week 12	7.98	9.76	0.288			
Week 16	8.48	9.49	0.288			
Isovalerate, m*M*/100 m*M*						
Mean	0.62	0.54	0.044	0.19	0.01	0.51
Week 12	0.55	0.50	0.054			
Week 16	0.69	0.58	0.054			
Valerate, m*M*/100 m*M*						
Mean	0.56	0.87	0.031	<0.01	0.23	0.70
Week 12	0.54	0.86	0.037			
Week 16	0.58	0.88	0.037			
Acetate:Propionate						
Mean	3.28	2.78	0.048	<0.01	0.53	0.42
Week 12	3.23	2.78	0.065			
Week 16	3.32	2.77	0.065			

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[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significance of effects for treatment (**Trt**), week (**Wk**), and treatment × week (**Trt × Wk**).

Table 16. Plasma metabolites and metabolic hormone concentrations for heifers limit-fed a control (**CON**) or distillers dried grains with solubles (**DDGS**) concentrate mix with ad libitum grass hay

	Treatment[1]			*P-value*[2]		
Item	CON	DDG	SEM	Trt	Wk	Trt × Wk
Cholesterol, mg/dL						
Mean	81.14	102.2	3.88	<0.01	0.20	0.45
Initial	96.00	96.90	3.76	0.77		
Week 4	80.74	94.55	4.99			
Week 8	83.53	107.0	4.99			
Week 12	81.75	104.2	4.99			
Week 16	78.52	102.9	4.99			
Glucose[3], mg/dL						
Mean	75.24	71.72	1.28	0.07	<0.01	0.81
Initial	81.10	81.66	1.51	0.63		
Week 4	72.63	70.35	1.66			
Week 8	74.51	70.41	1.66			
Week 12	76.21	71.74	1.66			
Week 16	77.60	74.38	1.66			
Insulin, ng/mL						
Mean	0.55	0.70	0.041	0.01	<0.01	0.15
Initial	0.49	0.56	0.045	0.01		
Week 4	0.39	0.43	0.062			
Week 8	0.51	0.62	0.056			
Week 12	0.66	0.93	0.060			
Week 16	0.64	0.81	0.060			
Plasma urea nitrogen, mg/dL						
Mean	12.49	11.59	0.31	0.06	0.49	0.64
Initial	14.49	14.58	0.80	0.87		
Week 4	12.92	11.48				
Week 8	12.24	10.66				
Week 12	11.75	12.01				
Week 16	13.07	12.23				
Triglycerides, mg/dL						
Mean	19.64	20.95	0.95	0.34	1.00	0.97
Initial	13.33	12.40	0.67	0.89		
Week 4	19.61	20.72	1.68			
Week 8	20.13	20.68	1.68			
Week 12	19.48	21.38	1.68			
Week 16	19.31	21.02	1.68			

[1] Control concentrate mix (**CON**), distillers dried grains with solubles (**DDG**) concentrate mix.

[2] Significance of effects for treatment (**Trt**), week (**Wk**), and treatment × week (**Trt × Wk**).

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

LITERATURE CITED

Abdelqader, M. M., A. R. Hippen, K. F. Kalscheur, D. J. Schingoethe, K. Karges, and M. L. Gibson. 2009a. Evaluation of corn germ from ethanol production as an alternative fat source in dairy cow diets. J. Dairy Sci. 92:1023-1037.

Abdelqader, M. M., J. L. Anderson, A. R. Hippen, D. J. Schingoethe, and K. F. Kalscheur. 2009b. In situ degradability of dry matter and protein from corn germ, distillers grains, and soybean meal. J. Dairy Sci. 92:2362 (Abstr).

Abeni, F., L. Calamari, L. Stefani, and G. Pirlo. 2000. Effects of gaily gain in pre- and postpubertal dairy heifers on body condition score, body size, and metabolic profile. J. Dairy Sci. 83:1468-1478.

Abrams, S. M., T. J. Klopfenstein, R. A. Stock, R. A. Britton, M. L. Nelson. 1983. Preservation of wet distillers grains and its value as a protein source for growing ruminants. J. Anim. Sci. 57:729-738.

Allain, C. C., L. S. Poon, C. S. G. Chan, W. Richmond, and P. C. Fu. 1974. Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470-475.

Anderson, J. L., D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2006. Evaluation of dried and wet distillers grains included at two concentrations in the diets of lactating dairy cows. J. Dairy Sci. 89:3133-3142.

Anderson, J. L., K. F. Kalscheur, A. D. Garcia, D. J. Schingoethe, and A. R. Hippen. 2009. Ensiling characteristics of wet distillers grains mixed with soybean hulls and evaluation of the feeding value for growing Holstein heifers. J. Anim. Sci. 87:2113-2123.

Anderson, J. L., K. F. Kalscheur, A. D. Garcia, and D. J. Schingoethe. 2015a. Feeding fat from distillers dried grains with solubles to dairy heifers: I. Effects on growth performance and total-tract digestibility of nutrients. J. Dairy Sci. 98:5699-5708.

Anderson, J. L., K. F. Kalscheur, J. A. Clapper, G. A. Perry, D. H. Keisler, A. D. Garcia, and D. J. Schingoethe. 2015b. Feeding fat from distillers dried grains with solubles to dairy heifers: II. Effects on metabolic profile. J. Dairy Sci. 98:5709-5719.

Anderson, J. L., K. F. Kalscheur, A. D. Garcia, and D. J. Schingoethe. 2015c. Short Communication: Feeding fat from distillers dried grains with solubles to dairy heifers: III. Effects on post-trial reproductive and lactation performance. J. Dairy Sci. 98:5720-5725.

Anderson, J. L., K. F. Kalscheur, A. D. Garcia, D. J. Schingoethe, D. P. Casper, and D. H. Kleinschmit. 2015d. Ensiling characteristics of distillers wet grains with cornstalks and determination of the feeding potential for dairy heifers. Prof. Anim. Sci.31:359-367.

AOAC International. 1995. Official Methods of Analysis. 16th ed. AOAC International, Arlington, VA.

AOAC International. 2002. Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists. Gaithersburg, MD.

Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. J. Dairy Sci. 88:E9-E21.

Bach Knudsen, K. E. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Anim. Feed Sci. Technol. 67:319-338.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method for total extraction and purification. Can. J. Biochem. Physiol. 39:911-917.

Block, S. S., J. M. Smith, R. A. Ehrhardt, M. C. Diaz, R. P. Rhoads, M. E. Van Amburgh, and Y. R. Boisclair. 2003. Nutritional and developmental regulation of plasma leptin in dairy cattle J. Dairy Sci. 86:3206-3214.

Butler, W. R. 1998. Review: Effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J. Dairy Sci. 81:2533-2539.

Carson, A. F., A. R. G. Wylie, J. D. G. Mc Evoy, M. Mc Coy, and L. E. R. Dawson. 2000. The effects of plane of nutrition and diet type on metabolic hormone concentrations, growth, and milk production in high genetic merit dairy herd replacements. Anim. Sci. 70:349-362.

Chalupa, W., B. Rickabaugh, D. S. Kronfeld, and D. Sklan. 1984. Rumen fermentation in vitro as influenced by long chain fatty acids. J. Dairy Sci. 67:1439-1444.

Chalupa, W., B. Vecchiarelli, A. Elser, D. S. Kronfeld, D. Sklan, and D. L. Palmquist. 1986. Ruminal fermentation in vivo as influenced by long-chain fatty acids. J. Dairy Sci. 69:1293-1301.

Chaney, A. L., and E. P. Marbach. 1962 Modified reagents for determination of urea and ammonia. Clin. Chem. 8:130-132.

Chelikani, P. K., D. J. Ambrose, D. H. Keisler, and J. J. Kennelly. 2009. Effects of dietary energy and protein density on plasma concentrations of leptin and metabolic hormones in dairy heifers. J. Dairy Sci. 92:1430-1441.

Chilliard, Y., M. Bonnet, C. Delavaud, Y. Faulconnier, C. Leroux, J. Djiane, and F. Bocquier. 2001. Leptin in ruminants. Gene expression in adipose tissue and mammary gland, and regulation of plasma concentration. Domest. Anim. Endocrinol. 21:271–295.

Christen, K. A., D. J. Schingoethe, K. F. Kalscheur, A. R. Hippen, K. K. Karges, and M. L. Gibson. 2010. Response of lactating dairy cows to high protein distillers grains or 3 other protein supplements. J. Dairy Sci. 93:2095-2104.

Day, M. L., K. Imakawa, M. Garcia-Winder, D.D. Zalesky, B.D. Schanbacher, R.J. Kittok, and J.E. Kinder. 1984. Endrocrine mechanism of puberty in heifers: estradiol negative feedback regulation of luteinizing hormone secretion. Biology of Reproduction 31:332-341.

Delavaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: Effect of nutrition status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165:519-526.

Diaz-Torga, G. S., M. E. Mejia, A. Gonzalez-Iglesias, N. Formia, D. Becu-Villalobos, and I. M. Lacau-Mengido. 2001. Metabolic cues for puberty onset in free grazing Holstein heifers naturally infected with nematodes. Theriogenology 56:111–122.

Engel, C. L., H. H. Patterson, and G. A. Perry. 2008. Effect of dried corn distillers grains plus soluble compared with soybean hulls, in late gestation heifer diets, on animal and reproductive performance. J. Anim. Sci. 86:1697-1708.

Enjalbert, F., J. E. Garrett, R. Moncoulon, C. Bayourthe, and P. Chicoteau. 1999. Effects of yeast culture (*Saccharomyces cerevisiae*) on ruminal digestion in non-lactating dairy cows. Anim. Feed Sci. Technol. 76:195-206.

Ettema, J. F., and J. E. Santos. 2004. Impact of age at calving on lactation, reproduction, health, and income in first-parity. Holsteins on commercial farms. J. Dairy Sci. 87:2730-2742.

Fahey, G.C., and L.L. Berger. 1988. Carbohydrate nutrition of ruminants. pages 269-295 in The Ruminant Animal: Digestive Physiology and Nutrition. D. C. Church, ed. Prentice Hall Inc., New Jersey.

Fossati, P., and L. Prencipe. 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem. 28:2077-2080.

Funston, R. N. 2004. Fat supplementation and reproduction in beef females. J. Anim. Sci. 82:E154-161.

Funston, R. N, J. L. Martin, D. M. Larson, and A. J. Roberts. 2012. Physiology and endocrinology symposium: Nutritional aspects of developing replacement heifers. J. Anim. Sci. 90:1166-1171.

Garcia, M. R., M. Amstalden, S. W. Williams, R. L. Stanko, C. D. Morrison, D. H. Keisler, S. E. Nizielski, and G. L. Williams. 2002. Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. J. Anim. Sci. 80:2158–2167.

Garcia, M. R., M. Amstanden, C. D. Morrison, D. H. Keisler, and G. L. Williams. 2003. Age at puberty, total fat and conjugated linoleic acid content of carcass, and circulating metabolic hormones in beef heifers fed a diet high in linoleic acid beginning at four months of age. J. Anim. Sci. 81:261-268.

Heinrichs, A. J. 1993. Raising dairy replacements to meet the needs of the 21st century. J. Dairy Sci. 76:3179-3187.

Hoffman, P.C., and D.A. Funk. 1992. Applied dynamics of dairy replacement growth and management. J. Dairy Sci. 75:2504-2516.

Hoffman, P. C. 1997. Optimum body size of Holstein replacement heifers. J. Anim. Sci. 75:836-845.

Hoffman, P. C., C. R. Simson, and M. Wattiaux. 2007. Limit-feeding of gravid Holstein heifers: Effect on growth, manure nutrient excretion, and subsequent early lactation performance. J. Dairy Sci. 90:946-954.

Hristov, A. N., R. P. Etter, J. K. Ropp, and K. L. Grandeen. 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. J. Anim. Sci. 82:3219-3229.

Jenkins, T. C. 1993. Lipid metabolism in the rumen. J. Dairy Sci. 76:3851-3863.

Kitts, B. L., I. J. H. Duncan, B. W. McBride, and T. J. DeVries. 2011. Effect of the provision of a low-nutritive feedstuff on the behavior of dairy heifers limit fed a high-concentrate ration. J. Dairy Sci. 94:940-950.

Kleinschmit, D. H., J. L. Anderson, D. J. Schingoethe, K. F. Kalscheur, and A. R. Hippen. 2007. Ruminal and intestinal degradability of distillers grains plus solubles varies by source. J. Dairy Sci. 90:2909-2918.

Klopfenstein, T. J., G. E. Erickson, and V. R. Bremer. 2008. Board invited review: Use of distillers by-products in the beef cattle feeding industry. J. Anim. Sci. 86:1223-1231.

Kutner, M. H., C. J. Nachtsheim, and J. Neter. 2004. Applied linear regression models. 4th ed. The McGraw-Hill Companies, Inc. New York, NY.

Lalman, D. L., J. E. Williams, B. W. Hess, M. G. Thomas, and D. H. Keisler. 2000. Effect of dietary energy on milk production and metabolic hormones in thin primiparous beef heifers. J. Anim. Sci. 78:530-538.

Lascano, G. J., and A. J. Heinrichs. 2009. Rumen fermentation pattern of dairy heifers fed restricted amounts of low, medium, and high concentrate diets without and with yeast culture. Livest. Sci. 124:48-57.

Leonardi, C., S. Bertics, and L. E. Armentano. 2005. Effect of increasing oil from distillers grains or corn oil on lactation performance. J. Dairy Sci. 88:2820-2827.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS for Mixed Models, 2nd ed. SAS Institute, Cary, NC.

Loerch, S. C., 1990. Effects of feeding growing cattle high-concentrate a restricted intake on feedlot performance. J. Anim. Sci. 68:3086-3095.

Loerch, S. C. 1996. Limit-feeding corn as an alternative to hay for gestating beef cows. J. Anim. Sci. 74:1211-1216.

Lussier-Cacan, S., E. Bolte', M. Bidallier, Y. S. Huang, and J. J. Daviguon. 1977. Cyclic fluctuations of plasma cholesterol in the female miniature swine and its relationship to progesterone secretion. Proc. Soc. Exp. Biol. Med. 154:471.

Maciel, M. N., D. A. Zieba, M. Amstalden, D. H. Keisler, J. P. Neves, and G. L. Williams. 2004. Chronic administration of recombinant ovine leptin in growing beef heifers: Effects on secretion of LH, metabolic hormones, and timing of puberty. J. Anim. Sci. 82:2930-2936.

Manthey, A. K., J. L. Anderson, and G. A. Perry. 2016. Feeding 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. J. Dairy Sci. accepted. http://dx.doi.org/10.3168/jds.2015-10785.

Martin, J. L., A.S. Cupp, R. J. Rasby, Z.C. Hall, and R.N. Funston. 2007. Utilization of dried distillers grains for developing beef heifers. J. Animal Sci. 85:2298-2303.

McFadden, T. B., T. E. Daniel, and R. M. Akers. 1990. Effects of plane of nutrition, growth hormone, and unsaturated fat on mammary growth in prepubertal lambs. J. Anim. Sci. 68:3171-3179.

Merchen, N.R. 1988. Digestion, Absorption and Excretion in Ruminants. pages 182-189 in The Ruminant Animal: Digestive Physiology and Nutrition. D. C. Church, ed. Prentice Hall Inc., New Jersey.

Meyer, M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006a. Development and nutritional regulation of the prepubertal heifer mammary gland: I. Parenchyma and fat pad mass and composition. J. Dairy Sci. 89:4289-4297.

Meyer, M. J., A. V. Capuco, D. A. Ross, L. M. Lintault, and M. E. Van Amburgh. 2006b. Developmental and nutritional regulation of the prepubertal bovine mammary gland: II. Epithelial cell proliferation, parenchymal accretion rate, and allometric growth. J. Dairy Sci. 89:4298-4304.

Mjoun, K., K. F. Kalscheur, A. R. Hippen, D. J. Schingoethe, and D. E. Little. 2010a. Lactation performance and amino acid utilization of cows fed increasing amounts of reduced-fat dried distillers grains with solubles. J. Dairy Sci. 93:288-303.

Mjoun, K., K. F. Kalscheur, A. R. Hippen, and D. J. Schingoethe. 2010b. Ruminal degradability and intestinal digestibility of protein and amino acids in soybean and corn distillers grains products. J. Dairy Sci. 93:4144-4154.

Moody, M. L., G. I. Zanton, J. M. Daubert, and A. J. Heinrichs. 2007. Nutrient utilization of differing forage-to-concentrate ratios by growing Holstein heifers. J. Dairy Sci. 90:5580-5586.

National Research Council. 2001. Nutrient Requirements of Dairy cattle. 7th Rev. Ed. Natl. Acad. Press, Washington, DC.

Nocek, J. E., and J. B. Russell. 1988. Protein and energy as an integrated system. Relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71:2070-2107.

Orth, R. 1992. Sample day and lactation report. DHIA 200 Fact Sheet A-2. Mid-states DRPC, Ames, IA.98.

Owens, F. N., and R. Zinn. 1988. Protein metabolism of ruminant animals. pages 227-249 in The Ruminant Animal: Digestive Physiology and Nutrition. D. C. Church, ed. Prentice Hall Inc., New Jersey.

Palmquist, D. L. 1981. Metabolite, insulin, and growth hormone concentrations in blood plasma of cows fed high fat diets for entire lactations. J. Dairy Sci. 64(Suppl 1):159 (Abstr.).

Palmquist, D.L. 1994. The role of dietary fats in efficiency of ruminants. J. Nutri. 124:1277S-1382S.

Park, C. S., G. R. Fisher, and C. N. Haugse. 1980. Effect of dietary protein and sunflower meal on blood serum cholesterol of dairy heifers. J. Dairy Sci. 63:1451-1454.

Park, C. S., W. Rafalowski, and G. D. Marx. 1983. Effect of dietary fat supplement on lipid metabolism on Holstein heifers. J. Dairy Sci. 66:528-534.

Perry, G. A. 2011. Harnessing basic knowledge of factors controlling puberty to improve synchronization of estrus and fertility in heifers. J. Anim. Sci. 90:1172-1182.

Ramirez-Ramirez, H. A., E. Castillo Lopez, C. J. R. Jenkins, N. D. Aluthge, C. Anderson, S. C. Fernando, K. J. Harvatine, and P. J. Kononoff. 2016. Reduced-fat dried distillers grains with solubles reduced the risk for milk fat depression and supports milk production and ruminal fermentation in dairy cows. J. Dairy Sci. 99:1912-1928.

Reynolds, C. K., H. F. Tyrrell, and P. J. Reynolds. 1991. Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers-Net nutrient metabolism by visceral tissues. J. Nutr. 121:1004-1015.

Robertson, J. B., and P. J. Van Soest. 1981. The detergent system of analysis and its application to human foods. Pages 123-158 in The Analysis of Dietary Fiber in Food. W.P.T. James and O. Theander, eds. Marcel Dekker Inc., New York, NY.

Ryan, D. P., B. Bao, M. K. Griffith, and G. L. Williams. 1995. Metabolic and luteal sequelae to heightened dietary fat intake in undernourished, anestrous beef cows induced to ovulate. J. Anim. Sci. 73:2086-2093.

Schillo, K. K., J. B. Hall, and S.M. Hileman. 1992. Effects of nutrition and season on the onset of puberty in the beef heifer. J. Animal Sci. 70:3994-4005.

Schingoethe, D. J., K. F. Kalscheur, A. R. Hippen, and A. D. Garcia. 2009. Invited review: The use of distillers products in dairy cattle diets. J. Dairy Sci. 92:5802–5813.

Schroer, R. C., T. D. Nennich, T. S. Dennis, M. M. Schutz, S. S. Donkin, and D. Little. 2014. Intake and growth of prepubertal dairy heifers fed reduced-fat dried distillers grains. Prof. Anim. Sci. 30:93-98.

Sejrsen, K., J. T. Huber, and H. A. Tucker. 1983. Influence of amount fed on hormone concentrations and their relationship to mammary growth in heifers. J. Dairy Sci. 66:845-855.

Sejrsen, K., J. T. Huber, H. A. Tucker, and R. M. Akers. 1982. Influence of nutrition of mammary development in pre-and postpubertal heifers. J. Dairy Sci. 65:793–800.

Sejrsen, K., and S. Purup. 1997. Influence of prepubertal feeding level on milk yield potential of dairy heifers: A review. J. Anim. Sci. 75:828-835.

Short, R. E., and R. A. Bellows. 1971. Relationships among weight gains, age at puberty, and reproductive performance in heifers. J. Anim. Sci. 32:127-131.

Sinha, Y. N., and H. A. Tucker. 1969. Mammary development and pituitary prolactin level of heifers from birth through puberty and during the estrous cycle. J. Dairy Sci. 52:507–512.

Suarez-Mena, F. X., G. J. Lascano, D. E. Rico, and A. J. Heinrichs. 2015. Effect of forage level and replacing canola meal with dry distillers grains with solubles in precision-fed heifer diets: Digestibility and rumen fermentation. J. Dairy Sci. 98:8054-8065.

Sukhija, P. S., and D. L. Palmquist.1988. A rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J. Agric. Food Chem. 36:1202-1206.

Susin, I., S. C. Loerch, K. E. McClure, and M. L. Day. 1995. Effects of limit-feeding a high grain diet on puberty and reproductive performance of ewes. J. Anim. Sci. 73:3206-3215.

Talavera, F., C. S. Park, and G. L. Williams. 1985. Relationships among dietary lipid intake, serum cholesterol, and ovarian function in Holstein heifers. J. Anim. Sci. 60:1045-1051.

Tamminga, S., C. J. Van Der Koelen, and A. M. Van Vuuren. 1979. Effect of the level of feed intake on nitrogen entering the small intestine of dairy cows. Livest. Prod. Sci. 6:255-262.

Thibault, C., D. Petitclerc, R. Spratt, M. Leonard, K. Sejrsen, and P. Lacasse. 2003. Effect of feeding prepubertal heifers with a high oil diet on mammary development and milk production. J. Dairy Sci. 86:2320-2326.

Thiex, N. 2009. Evaluation of analytical method for the determination of moisture, crude protein, crude fat, and crude fiber in distillers dried grains with solubles. J. AOAC Int. 92:61-73.

Thomas, M. G., B. Bao, and G. L. Williams. 1997. Dietary fats varying in their fatty acid composition differentially influence follicular growth in cows fed isoenergetic diets. J. Anim. Sci. 75:2512-2519.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen receptor. Ann. Clin. Biochem. 6:24-27.

Van Amburgh, M. E., D. M. Galton, D. E. Bauman, R. W. Everett, D. G. Fox, L. E. Chase, and H. N. Erb. 1998. Effects of three prepubertal body growth rates on performance of Holstein heifers during first lactation. J. Dairy Sci. 81:527-538.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wehrman, M. E., T. H. Welsh, Jr., and G. L. Williams. 1991. Diet-induced hyperlipidemia in cattle modifies the intrafollicular cholesterol environment, modulates ovarian follicular dynamics, and hastens the onset of postpartum luteal activity. Biol. Reprod. 45:514-523.

Wertz, A. E., L. L. Berger, D. B. Faulkner, and T. G. Nash. 2001. Intake restriction strategies and sources of energy and protein during the growing period affect sources of energy and protein during the growing period affect nutrient disappearance, feedlot performance, and carcass characteristics of crossbred heifers. J. Anim. Sci. 79:1598-1610.

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. J. Dairy Sci. 65:495-501.

Zanton, G. I., and A. J. Heinrichs. 2005. Meta-analysis to assess effect of prepubertal average daily gain of Holstein heifers on first-lactation production. J. Dairy Sci. 88:3860-3867.

Zanton, G.I., and A.J. Heinrichs. 2007. The effects of controlled feeding of a high-forage or high-concentrate ration on heifer growth and first-lactation milk production. J. Dairy Sci. 90:3388-3396.

Zanton, G. I., and A. J. Heinrichs. 2008. Rumen digestion and nutritional efficiency of dairy heifers limit-fed a high forage ration to four levels of dry matter intake. J. Dairy Sci. 91:3579-3588.

Zanton, G. I., and A. J. Heinrichs. 2009. Digestion and nitrogen utilization in dairy heifers limit-fed a low or high forage ration at four levels of nitrogen intake. J. Dairy Sci. 92:2078-2094.

Zieba, D. A., M. Amstalden, S. Morton, M. N. Maciel, D. H. Keisler, and G. L. Williams. 2004. Regulatory roles of leptin at the hypothalamic-hypophyseal axis before and after sexual maturation in cattle. Biol. Reprod. 71:804-812.

Zieba, D. A., M. Amstalden, and G. L. Williams. 2005. Regulatory roles of leptin in reproduction and metabolism: A comparative review. Domest. Anim. Endocrinol. 29:166-185.